

Response of *Chilo partellus* (Lepidoptera: Crambidae) to Bt maize in South Africa

J Vorster

 orcid.org/0000-0001-8126-6860

Dissertation submitted in fulfilment of the requirements for the *Masters* degree in *Environmental Science* at the North-West University

Supervisor: Prof J van den Berg
Co-supervisor: Prof MJ du Plessis
Assistant supervisor: Dr A Erasmus

Graduation **May 2018**

23441674



ACKNOWLEDGEMENTS

This dissertation would not have been possible without the help of so many people. I am blessed and very grateful to have them in my life. I would like to start with our God Almighty and our Saviour who bestowed upon me the strength, wisdom and peace of mind to finish this project and who also have sent me these blessed people in my life.

I would like to thank Prof. Johnnie van den Berg and Dr. Annemie Erasmus for all the guidance and support they have given me. You taught me that small things can make a big difference.

Statistics can be difficult sometimes and I thank Prof. Hannalene du Plessis and Prof. Suria Elis for the help with the statistics.

Thank you to all the staff at the ARC-GCI that assisted me with the trials in the lab and the planting. Elrine Strydom, Mabel du Toit, Heidi Meyer and Ursula du Plessis, thank you for the countless after hours we had to spend and for the warm hearted kindness you have given me.

I would also like to thank my parents whom I dearly love for all the encouragement and motivation to do my best. You taught me that hard work does not come easily, but the fruit that you pick from it is what motivates us.

To my fiancé Fredry, thank you for being so patient and for all the love and support you have given me. Thank you for countless hours you had spent with me and motivation you have given me to finish this project.

ABSTRACT

Maize is an important food resource for humans and their livestock. South Africa is one of the top maize producing countries and also one of the largest producers of genetically modified maize in the world. Maize pests provide important challenges to the sustainable production of maize in Africa and pests such as *Busseola fusca* (Lepidoptera: Noctuidae), and *Chilo partellus* (Lepidoptera: Crambidae), pose a serious threat to maize production since borer damage contributes to yield losses and poor grain quality. Bt maize was developed to target lepidopteran stem borers and *C. partellus* has been effectively controlled by Bt maize since its cultivation commenced during 1998. Over the years several Lepidoptera pests developed resistance to transgenic Bt crops. In South Africa, the African maize stem borer, *B. fusca* developed resistance to the MON810 Bt event, only eight years after the first release of Bt maize. However, the spotted stem borer *C. partellus* has not developed resistance to Bt maize in South Africa. The non-compliance to refuge requirements as part of an insect resistance management strategy contributed to resistance evolution of *B. fusca* and should therefore also have resulted in relatively quick resistance evolution in *C. partellus*. The aims of this study were to determine how *C. partellus* moths and larvae respond towards Bt maize in choice and no-choice tests and to determine the effect of larval size and survival on Bt maize. An attempt was also made to select for resistance in a population under laboratory conditions. Different sizes of larvae of four populations were used in survival studies and it was observed that older larvae were able to survive on Bt maize tissue. It was possible to select for tolerance to Bt over a two generation life cycle of this pest by allowing neonate larvae to feed on Bt maize tissue for short periods of time and then allowing the survivors to complete their life cycles on non-Bt maize. This study showed that *C. partellus* moths did not exhibit any oviposition preference towards the Bt or non-Bt treatments used in this study. This study concluded that if neonate larvae that hatch on a natal Bt maize plant move off the Bt maize plant onto a non-Bt plant within 24 hours, larvae will be able to survive. This study further concluded that *C. partellus* is still highly susceptible to Bt maize in South Africa and that behavioural characteristics such as larval movement between plants, may contribute to resistance evolution.

Keywords: Bt maize, *Chilo partellus*, preferences, resistance development, resistance management

Table of Contents

ACKNOWLEDGEMENTS	ii
ABSTRACT	iii
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW	1
1.1 Maize production	1
1.2 The Chilo borer.....	1
1.3 Distribution of <i>Chilo partellus</i>	2
1.4 Life cycle of <i>Chilo partellus</i> and damage caused by larvae	2
1.4.1 Life cycle of <i>Chilo partellus</i>	2
1.4.2 Damage symptoms and yield losses.....	7
1.5 Management of stem borers	9
1.6 Aim and objectives.....	16
1.7 References	16
CHAPTER 2: THE STATUS OF RESISTANCE AND EFFECT OF LARVAL SIZE ON SURVIVAL OF <i>CHILO PARTELLUS</i> ON BT MAIZE IN SOUTH AFRICA	26
2.1 Abstract	26
2.2 Introduction.....	27
2.3 Materials and methods	28
2.3.1 Laboratory bioassay.....	28
2.4 Results	30
2.4.1 Evaluation of larval survival and growth	30
2.4.2 Evaluation of pupal development	40
2.5 Discussion	42
2.6 Conclusions.....	43
2.7 References	43
CHAPTER 3: THE EFFECT OF <i>CHILO PARTELLUS</i> LARVAL AGES ON BT MAIZE	45
3.1 Abstract	45
3.2 Introduction.....	46
3.3 Materials and methods	47
3.3.1 Parental generation (F0 generation).....	47
3.3.2 F1 generation.....	48

3.4 Results	51
3.5 Discussion	58
3.6 Conclusions	59
3.7 References.....	59
CHAPTER 4: THE PREFERENCE OF CHILO PARTELLUS MOTHS, AND LARVAE FOR BT MAIZE AND NON-BT MAIZE	61
4.1 Abstract	61
4.2 Introduction.....	62
4.3 Materials and methods	63
4.3.1 Preference of inexperienced neonate larvae	63
4.3.2 Preference of experienced neonate larvae.....	67
4.3.3 Oviposition preference	68
4.4 Results	69
4.4.1 Preference of inexperienced neonate larvae	69
4.4.2 Preference of experienced neonate larvae.....	73
4.4.3 Oviposition preference	73
4.5 Discussion	74
4.6 Conclusions	75
4.7 References.....	76
CHAPTER 5: CONCLUSIONS	78
5.1 References	80

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Maize production

Maize is one of the most important food resources in the world, because it is utilised in various ways such as for human consumption, animal feed or industrial ways and biofuel. During 2015/16 an area of 177.76 million hectares in maize was planted globally and of which there was an average yield of 5.41 tons per hectare (FAS/USDA, 2017). Although this is a high yield, the amount of maize is still not enough for the growing population, especially in developing countries. In Africa 95% of the maize that is produced is used for human food (Ntiri *et al.*, 2016). In Africa a total of 37 million hectares of maize was harvested (2.1 metric tons per hectare), yielding 78 million tons in 2014 (FAO, 2017). In South Africa there is an estimated 1.9 million hectares of maize planted during 2016 and a total of 6.4 million tons was harvested (SAGIS, 2017). According to the statistics of SAGIS (2017) this was a 26.6% decrease from 2015 and since South Africa was one of the top 10 maize producing countries in the world in 2014, it causes great concern. Challenges such as drought, diseases, nutrient deficiency and pests may pose a threat to sustainable maize production (Ntiri *et al.*, 2016).

1.2 The Chilo borer

In South Africa one of the most important challenges is the management of insect pests in maize, for example *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *Chilo partellus* was first described by Colonel Charles Swinhoe in 1885 as *Crambus partellus* and was found in large numbers in Poona in India (Swinhoe, 1885; Kalaisekar, 2017). Later Bleszynski (1970) named *Chilo zonellus* a synonym of *C. partellus* in a thorough revision of all known *Chilo* species. There are a number of *Chilo* spp. that attack crops in Africa and nearby islands. In the Indian Ocean Islands, the stem borer *Chilo sacchariphagus* (Bojer) is an important pest of sugarcane (Kfir *et al.*, 2002). In the coastal areas of East Africa *Chilo orichalcociliellus* (Strand) is a pest of sorghum and maize (Kfir *et al.*, 2002). In West and Central Africa, *Chilo aleniellus* (Strand) is a pest of rice, but in the Ivory Coast it is an important pest of maize (Kfir *et al.*, 2002). Of all the *Chilo* spp, *C. partellus* is the most economically important in Africa (Kfir *et al.*, 2002).

1.3 Distribution of *Chilo partellus*

Chilo partellus is native to Asia and its distribution includes Yemen, Vietnam, Thailand, Sri-Lanka, Pakistan, Nepal, Laos, Iran, Indonesia, India, Cambodia, Bangladesh and Afghanistan (Yonow *et al.*, 2016). *Chilo partellus* was recently also reported in Turkey's Mediterranean region and in Israel, where reports indicated its presence in Western Galilee on sorghum and maize plants (Ben-Yakir *et al.*, 2013, Bayram & Tonga, 2015). *Chilo partellus* must have invaded Africa during the 1920's since it had been already recorded in Malawi at that time. Since then it has spread to other African countries such as Zimbabwe, Zambia, Uganda, Tanzania, Swaziland, Sudan, South Africa, Somalia, Mozambique, Lesotho, Kenya, Ethiopia, Eritrea, the Comoros Islands and Botswana (Kfir *et al.*, 2002; Yonow *et al.*, 2016).

The first record of *C. partellus* in South Africa was in 1958 in sorghum on the Springbok Flats (Van Hamburg, 1971). The first collection of *C. partellus* larvae in South Africa was done by A. Barnard on 12 March 1958 near Naboomspruit (Van Hamburg, 1979). In 1974 it was discovered in Potchefstroom (Van Hamburg, 1976). These species also occur in mixed populations with the African maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and in grain sorghum *C. partellus* was reported to be more injurious than *B. fusca* (Van den Berg *et al.*, 1991).

The following crops and or plants are the hosts for *C. partellus*, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), rice (*Oryza sativa*), Pearl millet (*Pennisetum glaucum*) and grasses such as Sudan grass (*Sorghum vulgare sudanense*) (Hutchinson *et al.*, 2007; Khan *et al.*, 2006; Van den Berg, 2006).

1.4 Life cycle of *Chilo partellus* and damage caused by larvae

1.4.1 Life cycle of *Chilo partellus*

Eggs

Chilo partellus can oviposit a mean number of 434 eggs per female (Ofomata *et al.*, 2000) (Berger, 1989). According to studies done by Van Hamburg (1971) the female moths can lay a maximum of 563 eggs. According to Hutchinson *et al.* (2008) moths prefer to lay eggs on plants during the vegetative growth stages of maize. Oviposition occurs over two to four nights (Berger, 1989). The eggs appear as yellow flecks on the maize leaves. The eggs are flat, slightly oval in shape and creamy, yellow in colour (Deep & Rose, 2014). After two days the

eggs become yellowish in colour and on the third day they become yellowish brown (Siddalingappa *et al.*, 2010). The eggs hatch five to six days after oviposition (Panchal & Kachole, 2013) and are usually laid at night (Kalaisekar *et al.*, 2017).



Figure 1-1: An egg batch of *Chilo partellus* (Photo by A. Erasmus)

Larvae

After hatching larvae migrate to the whorl of the maize plant, where larvae establish inside the funnel leaves (Berger, 1992). The larvae do not feed before they establish inside the whorl (Berger, 1989). Some neonate larvae will also balloon with silk threads from the maize plant. This behaviour is important for the larvae as it makes transportation via wind to other plants possible (Berger, 1989). When larvae feed on the maize whorls they cause small holes (pin holes) and may scar the leaf epidermis (Sithole, 1990). As larvae mature in the whorl the growing points may be destroyed resulting in the typical dead heart symptoms where the central leaves die in young host plants (Sithole, 1990; Kumar, 1997). As soon as larvae reach second instar stage, a second dispersal phase occurs when they climb out of maize whorls and may balloon away or move to other plants (Berger, 1992). During the third dispersal phase the larvae will migrate from the whorl down outside the stem and seek new feeding sites among identical plants, sometimes within the stem (Berger, 1992). The late departure from the plant's whorl, occurs throughout the first and second week after the eggs hatch, this is also followed by a migration of larvae between plants (Berger, 1992). Stem tunnelling causes severe damage to the plants and this causes the maize plant to weaken, subsequently reducing water, nutrient

and metabolite transportation through the plant (Sylvain *et al.*, 2015). According to Kumar and Saxena (1994) foliar damage and stem tunnelling are the most important parameters for comparing the intensity of *C. partellus* damage especially for different cultivars to test their susceptibility to this pest. During the last dispersal phase, which starts from the second week after egg hatch until pupation, the third-instar or older larvae will leave the plant and walk on the ground or onto plant leaves of other plants (Berger, 1992). Here, they will then bore into the stems and pupate. The fully grown larvae are about 25-30 mm in length, have a black or dark brown head and appear creamy white in colour with rows of dark spots on the body (Panchal & Kachole, 2013). The total duration of larval period of *C. partellus* is 20 to 51 days (Siddalingappa *et al.*, 2010).



Figure 1-2: A fifth instar larva of *Chilo partellus*

Pupae

The pupae are obtect, which means that it is embodied in a hard case with the legs and the wings attached immovably (Kalaisekar *et al.*, 2017). Pupae are dark brown in colour and about 12 mm long (Panchal & Kachole, 2013). Studies done by Pedda Kasim *et al.* (2016) found that the sizes of the female pupa (1.6 ± 0.05 cm) are slightly larger than male pupa (1.21 ± 0.02 cm). The pupal stage lasts approximately five to 10 days (Panchal & Kachole, 2013). Before fully grown larvae would pupate, they would eat an exit hole into the stem, which ensure that the moths can leave after emergence (Kalaisekar *et al.*, 2017).



Figure 1-3: The pupa of *Chilo partellus*

Moths

Chilo partellus moths are pale brown in colour with a wingspan of 20 - 30 mm (Panchal & Kachole, 2013). Females are much lighter in colour than the males which have pale brown forewings and white hind wings (Panchal & Kachole, 2013). Female moths are slightly larger than the males. The male moth has an average length of 1.36 ± 0.05 cm and the female moth an average of 1.70 ± 0.03 cm (Pedda Kasim *et al.*, 2016). The moths emerge usually in the afternoon or early in the evening, taking refuge under stones and plant residues during the day (Siddalingappa *et al.*, 2010; Kalaisekar *et al.*, 2017). After the females emerge and males find them, mating takes place (Kalaisekar *et al.*, 2017). According to studies done by Pedda Kasim *et al.* (2016) the male moths live for three to seven days and the females for three to eight days. The female moths prefer to oviposit on plants during the vegetative growth stages (Hutchinson *et al.*, 2008).

According to observations made at the KARI-Katamani laboratory in Kenya, *C. partellus* undergoes approximately 11 to 12 generations in a year (Mutisya *et al.*, 2013) when reared on artificial diet. If climate conditions are optimal, there may be 11 to 12 generations in regions where the climate is optimal for development and do not vary much over time. *Chilo partellus* remains active throughout the year in Africa (Kumar, 1997). In the beginning of September to mid-December in southern Africa, the first generation moths start to fly and there can be up to five generations per cropping season. After harvest, larvae may overwinter in maize stubble in the cooler climate regions of southern Africa (Van Hamburg, 1979).



Figure 1-4: The female (left) and male (right) moths of *Chilo partellus*

Diapause

Chilo partellus diapauses in winter in mainly the lower parts of dry maize and sorghum stubbles (Kfir, 1993). Diapause is defined by Gilbert (2005) as a type of dormancy that involves perception of one or more predictable environmental cues that anticipate and predict regularly occurring, unfavourable conditions. In the case of *C. partellus* the diapause induction is caused by dry conditions as well as the degeneration of the nutritional environment of its host plants (Kfir, 1993). Gilbert (2005) stated that diapause is the process by which the neuroendocrine system controls a genetically determined response and that it can either be obligate or facultative. However, *C. partellus* diapause is seen facultative, since the larvae do not diapause in all areas of its distribution (Kfir, 1993). When the larvae enter diapause, they become less active and lose pigmentation. *Chilo partellus* larvae become white as the larvae lose their spots. This is due to the loss of energy reserves in the form of fat that larvae accumulated before diapause commenced (Kfir, 1991). However, Kfir (1991) found that not all *C. partellus* larvae that enter diapause may lose their non-diapause appearance and they can therefore not be distinguished on the basis of loss of their pigmentation. Studies by Kfir (1991) found that *C.*

partellus can have up to six stationary molts during diapause and that only 10% of larvae have more than three stationary moults. Kfir (1991) found that the female moths of diapause larvae weighed less and contained fewer eggs compared to those of non-diapausing larvae. Kfir (1991) ascribed this to moulting which results in larvae losing weight when they use the energy reserves. Therefore, male and female moths emerging from pupae that formed from diapause larvae are smaller and they also weigh less. Kfir (1993) found that diapause termination is influenced not only by temperature but also water availability and photoperiod. Kfir (1991) concluded that less severe pest infestations occur during the beginning of the cropping season in September and he ascribed this to the poorer quality first generation moths that emerge from diapause larvae; these moths then have low fecundity. Higher levels of infestation occur later in the cropping season due to the higher fecundity of moths from the second generation.

1.4.2 Damage symptoms and yield losses

Damage caused by *C. partellus* to maize is similar to damage caused to sorghum. In the Peshawar valley of Pakistan, *C. partellus* causes damage ranging between 24 and 75% (Can Cengiz *et al.*, 2016). In Mozambique, the third generation of *C. partellus* have been reported to infest up to 87% of ears of late-planted maize, resulting in yield losses of up to 70% (Kfir *et al.*, 2002). According to Kfir *et al.* (2002), studies showed that commercial farms have less than 30% of borer infestation in maize while infestation levels in maize in resource-poor farming areas range between 30 and 70%. This difference is ascribed to the large scale use of Bt maize and insecticides in commercial farming systems.

Damage symptoms

There are three types of damage symptoms namely foliar lesions (whorl damage), stem tunnelling and the 'dead heart' symptoms which occur during the pre-flowering stages of maize.

Whorl damage

After eggs hatch, neonate larvae migrate to the whorl of the maize plant. There larvae establish and start feeding on the maize whorl leaves. This feeding causes lesions or small holes that appear as pin holes as the whorl leaves unfold (Ajala & Saxena, 1994; Sithole, 1990).

'Dead heart'

'Dead heart' symptoms occur when larvae feed in the maize whorl and the growth point is damaged to such an extent that the whorl leaves die off (Razig & Ishag, 2014).

Stem tunnelling

After the larvae leave maize whorls they migrate downwards to penetrate the maize stem or move to neighbouring plants. After stem penetration, the larvae create tunnels through the vascular bundles, which then reduces translocation of nutrients (Razig & Ishag, 2014). Furthermore, stem tunnelling causes the reduction in the vitality of the plant and the grain filling process. Stem damage caused by larvae also promotes breakage and the lodging of plants as they mature (Sylvain *et al.*, 2015).

Ear damage

Chilo partellus also feeds on maize ears. Larval damage deforms the ears and reduces grain quality. However, the greatest concern is that this physical damage to ears creates a suitable environment for infection by fungi such as *Fusarium* spp. which could then lead to the production of mycotoxins (Sylvain *et al.*, 2015).

Yield losses

Stem borers can cause yield loss of between 10% or total loss (Kfir *et al.*, 2002; Obonyo *et al.*, 2008). In Kenya stem borers cause yield losses ranging between 13 and 50% (Tende *et al.*, 2005). In Ethiopia stem borers such as *B. fusca* and *C. partellus* have been reported to cause losses of between 25 and 100% (Belay & Foster, 2010). According to Wahedi *et al.* (2016) stem borer damage to maize plants can range between 20 and 40 % losses during cultivation. Grain yield losses from *C. partellus* damage to maize do however differ between cultivars, the plant growth stage at which the attack commences, infestation levels and nitrogen application rates (Kumar, 1997; Kumar & Saxena, 1992; Mashwani *et al.*, 2015; Mgoo *et al.*, 2006).

In Nepal yield loss caused by *C. partellus* have been estimated to be between 20 and 87% (Neupane *et al.*, 2016). *Chilo partellus* is also a serious pest in India where yield losses between 26.7 and 80.4% have been reported in different agro-climatic areas (Hari *et al.*, 2008; Pedda Kasim *et al.*, 2016). In Africa these stem borers can cause yield losses as high as 88% in maize (Can Cengiz *et al.*, 2016). *Chilo partellus* can cause yield losses of between 50 and 60% in

sorghum in Zimbabwe (Kfir *et al.*, 2002). In South Africa losses due to *C. partellus* in maize and sorghum have been reported to be in the range of 50% in the North West and Free State provinces (Sithole, 1990). In order to limit yield losses caused by *C. partellus*, sound pest management strategies, based on economic injury levels should be employed.

Economic injury levels

The economic injury level (EIL) is the lowest population density which will cause economic injury (Stern *et al.*, 1959). Seshu Reddy and Sum (1991) recommended that if a mean infestation level of 3.2 and 3.9 *C. partellus* larvae occur per plant during between 20 and 40 days after seedling emergence, that measures needed to be taken to ensure economic control of maize. Therefore, infestations occurring at an early growth stage may result in severe crop losses. This was later confirmed by Van den Berg and Van Rensburg (1991) as well as Bate and Van Rensburg (1992). Van den Berg and Van Rensburg (1991) did studies on the infestation and injury levels of stem borers in grain sorghum and found that infestation occurs from three weeks after seedling emergence onwards and that it may continue to increase until boot stage. Bate and Van Rensburg (1992) also confirmed this in the studies they did on maize with *C. partellus*. Natural infestation occurs at 3 to 5 weeks after emergence and a second infestation may occur from the tasseling stage onwards. Bate and Van Rensburg (1992) reported that 40% of plants exhibiting whorl damage symptoms is an appropriate action threshold for control of this pest on maize.

1.5 Management of stem borers

Integrated pest management

The over use of chemicals to control pests resulted in resistance development, pest outbreaks, pollution of the environment and health problems (Abrol & Shankar, 2012). Due to the failure of organic synthetic insecticides, the concept of integrated pest control gained more popularity. (Kogan, 1998). According to Pimental and Peshin (2014) integrated pest management (IPM) is a system that takes into account the environment and the population dynamics of pest species and then use of strategies suitable to maintain pest populations below levels that cause economic losses. These four pillars of integrated pest management are: cultural control, biological control, host plant resistance and chemical control.

Cultural control of stem borers

Cultural control is the manipulation of the environment to render it unsuitable or unfavourable for the pest (Dent, 1991). This method helps by interfering in pest colonization of a crop, the promotion of dispersal or even reducing reproduction and survival of the pest. Basic examples of such techniques include crop rotation, intercropping, planting date manipulation, and destruction of crop residues through burning or tillage and habitat management. Other interference methods include semiochemical usage to disrupt insect communication and the sterile insect technique. According to Dent (1991) cultural control should be the prime control method that other control methods should be built on. Currently, this is the best method for stem borer control for resource poor farmers in Africa (Kfir *et al.*, 2002).

Crop rotation

Crop rotation is the sequence of different crops that are grown or planted on the same field over time. The practice of crop rotation gives positive effects from the one crop to the next. Brankatsck and Finkbeiner (2015) listed the following advantages of crop rotation:

- Reduced agrochemical and synthetic fertilizer usage
- Facilitates the proper timing of farming activities
- Improvement of soil structure
- Improvement of soil texture
- Higher yields and improved soil fertility
- Maintenance of long-term productivity and increased organic matter content in soils
- Improvement of the population of micro-organisms
- Weed seed reduction
- Increased biodiversity
- Lower economic and climatic risks
- Greater market opportunities.

Crop rotation is however only effective against pest species if the pest has a narrow host range and a limited range of dispersal. According to Dent (2000) the main objective of crop rotation is to reduce pest colonization by planting a non-host crop species during the follow-up season. This forces pest to disperse and if they are poor dispersers, they will not be able to find a host crop. Dent (2000) provided an example of the Colorado potato beetle where potato and wheat crops are rotated. The crops are rotated each year and in this case the potatoes are planted in

adjacent fields that are separated from other crops. This distance helped also as this beetle pest only disperses by walking to the over wintering sites (Dent, 2000).

Crop rotation is not always effective and in the case of *C. partellus* it would be less effective as it has a wider host range than *B. fusca*. Other cultural methods must therefore be used for the management of this stem borer species.

Intercropping

One of the problems we face today in agriculture is the use of monocultures. In tropical countries where farms are often smaller, traditional approaches such as multi-cropping and intercropping are often practised (Dent, 2000). Polycultures include mixed cropping (randomised and no rows), row intercropping (crops planted in rows of one or more variation), strip intercropping (crops are planted in strips with enough space for cultivation), relay intercropping (two or more crops grown at the same time, but second crop is planted at the time of harvest of the first crop) and alley intercropping (crops that are planted between trees (Dent, 2000). Some of the benefits of intercropping are the reduction in the risk of crop failure, contributing to higher yields and soil improvement (Kfir *et al.*, 2002). According to Maluleke *et al.* (2005) in areas where maize and sorghum are planted, these two crops usually are intercropped with other non-host crops which reduce the opportunity for pests to reach outbreak status in these fields. An example of a non-host intercrop of *C. partellus* is legumes such as *Lablab purpureus* that is planted for its foliage, seed and nitrogen-fixing properties (Maluleke *et al.*, 2005). Studies shown by Skovgård and Pats (1997) on intercropping cowpea with maize reduced the damage done by stem borers significantly and also increased the maize yield, but Skovgård and Pats (1997) concluded that this would not be enough to control stem borers. According to the studies of Maluleke *et al.* (2005) intercropping with legumes, to control stem borers such as *C. partellus*, may also have a negative effect on the crop yield if the different crops that are planted at the same time.

Planting date

The best way to ensure that the most susceptible growth stage of crop is not damaged by a pest, is to plant the crop during a period that the adult individuals, which lay eggs, are not in their peak activity period (Kfir *et al.*, 2002). According to Van Hamburg (1979), the largest and most fecund *C. partellus* moths emerge during February to April and if planting occurred during this time there would be *C. partellus* infestations. Van Hamburg (1979) reported that infestation

before the boot stage (approximately 55 days after planting) of sorghum results in significant yield losses, but that later infestations are not economically important. For this reason, sorghum should be planted after mid-October (to avoid first moth peak) and before mid-December (to avoid the second moth peak) in South Africa. Thus the time or date when crop plants are planted has a significant effect on crop yield (Dent, 2000). Studies by Van den Berg and Van Rensburg (1991) confirmed the results of Van Hamburg (1979) that planting date was the only factor which had a significant effect on *C. partellus* infestation levels.

Destruction of crop residues (tillage or burning)

Stem borers overwinter in crop residues. According to Kfir *et al.* (2002) ploughing to destroy overwintering larvae was one of the effective measures to control *B. fusca* in South Africa and is still important today. *Busseola fusca* and *C. partellus* overwinter in maize and sorghum stems, and about 90 000 and 226 000 larvae per hectare may overwinter on these crops respectively. Kfir (2002) reported that destroying / slashing of crop residues destroyed 70% of *C. partellus* and *B. fusca* populations and if ploughing was also done, another 24% of the pest population in sorghum and 19% in maize are destroyed (Kfir *et al.*, 2002). By using tillage, the larvae or pupae are buried deep underneath the ground that prevents the moths from emerging, or the stubbles containing larvae are exposed to the harsh environments and natural enemies (Kfir *et al.*, 2002). Even though tillage is an effective control measure, many farmers today are implementing conservational tillage and this is due to the impact that tillage practices have on labour costs, machinery wear, soil erosion and soil moisture content (Dent, 2000). Another control measure is to burn crop residues. According to Kfir *et al.* (2002) burning of crop residues in Tanzania have almost eradicated *C. partellus*. However, burning of crop residues cause problems for fields that have a low organic soil content and may lead to increased erosion.

Habitat management

Another effective control measure is the use of trap cropping and push-pull systems. Trap plants are usually hosts that are highly preferred for oviposition and is planted around the crop (Shelton & Badenes-Perez, 2006). In Africa a high diversity of grass species in the tropical areas that surround farmers' fields is an essential control measure for the management of stem borers (Khan *et al.*, 2006). Khan *et al.* (2006) found that *C. partellus* had a higher oviposition preference for Napier grass varieties than maize. Furthermore, the use of Napier grass (*Pennisetum purpureum*) as trap plant around maize plots in push-pull systems helped to

reduce stem borer infestations in maize crops (Khan *et al.*, 2006). Recent studies done by Van den Berg and Van Hamburg (2015) found that *B. fusca* had the same preference for Napier grass and maize for oviposition and it is therefore not effective as a trap plant for *B. fusca*. On the other hand, *C. partellus* does have a stronger oviposition preference for Napier grass than for maize (Khan *et al.*, 2006). Napier grass is not the only trap crop plant for *C. partellus*. Vetiver grass (*Vetiveria zizanioides*) is also an effective trap plant and according to Van den Berg (2006), Vetiver grass is not preferred by *B. fusca* for oviposition but *C. partellus* prefers Vetiver grass over maize to oviposit their eggs. Despite the preference of moths for Vetiver grass, hardly any larvae survive on this grass, making it an ideal trap crop (Van den Berg, 2006).

Biological control of stem borers

Biological control is still an effective control measure today since it was first practiced during the 1800's. This approach has fewer risks involved and also provides a cost-effective solution (Dent, 2000). In order for biological control to be successful, the target pest must always be present in adequate numbers and also at a suitable life stage at the release sites. At the release site certain factors need to be taken in consideration such as the following: optimal conditions, the number of natural enemies needed, especially in the target pest geographical or ecological range (Dent, 2000). Biological control includes the use of parasitoids, nematodes and viruses or pathogens.

In South Africa, *C. partellus* has several parasitoid species that attack the larval and pupal stages, but these species fail to reduce densities to below economic damaging levels (Kfir, 1994). Biological control is employed as a method to control stem borers since insecticides are often not effective and too expensive (Kfir, 1994). According to Kfir (1990) more *C. partellus* larvae on the maize was parasitized than on sorghum, where larval parasitism was at 66% and pupal parasitism 67% on maize and less on sorghum. No parasitoids have been recorded during the winter season on the South African high veld but some parasitoids may hibernate within the stem borer larvae in maize stubbles (Kfir, 1990).

Table 1-1: List of parasitoids that parasitizes on *Chilo partellus* (Kfir, 1990; Skoroszewski & Van Hamburg, 1987; Van Hamburg, 1984).

Parasitoid	Order: family	Type of parasite	Notes
<i>Cotesia flavipes</i>	Braconidae	Larval parasite	Indigenous to south and south east Asia. Three to 13% parasitism of <i>C. partellus</i> .
<i>Cotesia sesamiae</i>	Braconidae	Larval parasite	Larvae that emerge spin cocoons and up to 70 % of parasites can develop in 1 host. Can cause 93% of parasitism of <i>C. partellus</i> . Recorded in Ethiopian region.
<i>Bracon spp.</i>	Braconidae	Ectoparasite	Develops on older larvae. About 30 can develop in its host. Rare parasite of <i>C. partellus</i> and active during winter
<i>Chelonus curvimaculatus</i>	Braconidae	Egg-larval solitary endoparasite	Rare parasite. Attacks the eggs of <i>C. partellus</i> and emerges from older larvae. Also found on other insect pests in South Africa.
<i>Chelonus spp.</i>	Braconidae	Egg-larval solitary endoparasite	Rare parasite of <i>C. partellus</i> .
<i>Conomorium spp.</i>	Pteromalidae	Gregarious pupal parasite	Rare parasite that attacks <i>C. partellus</i> . About 30 to 50 parasites can develop in one host
<i>Dentichasmias busseolae</i>	Ichneumonidae	Pupal parasite	Important parasite of <i>C. partellus</i> in Africa and other regions. Lays only a single egg inside pupa. Can cause 100% parasitism.
<i>Iphiaulax spp.</i>	Braconidae	Larval parasite	Uses antennae to locate <i>C. partellus</i> larvae. Lays single egg and consumes host and spin into cocoon. Can hibernate in their cocoon.
<i>Norbanus spp.</i>	Pteromalidae	Larval parasite	Rare parasite that parasitizes in winter on full grown <i>C. partellus</i> larvae. A max of 8 parasites develop in one host.
<i>Pediobius furvus</i>	Eulophidae	Pupal parasite	Indigenous to Africa. Can affect up to 67% parasitism of <i>C. partellus</i> in South Africa. About 250 parasitoids may develop in one host.
<i>Pristomerus spp.</i>	Ichneumonidae	Solitary endoparasite	Attacks <i>C. partellus</i> larvae late during the cropping and hibernates inside stem borer larvae during winter.
<i>Trichogrammatoidea lutea</i>	Trichogrammatidae	Egg parasite	Recorded in Ethiopia, Ivory Coast and Kenya. Attacks the eggs of stem borers. Can cause 50% reduction in numbers of last-instar larvae.

Host plant resistance to stem borers

Host plant resistance the inherent ability of a plant to restrict or retard or even to overcome pest infestations and then also to improve not only the quality but also the quantity of the yield of a harvestable crop product (Dent, 2000). According to Munyiri *et al.* (2015), the effects that resistant plants may have on herbivorous insects are antibiosis, antixenosis. Antibiosis is the effect that plants may have on pest biology namely its survival, development, reproduction and also their fitness parameters. Antixenosis is when a plant has mechanisms that cause the insect not to prefer the plant, thereby inducing changes in pest behaviour, orientation, oviposition and even feeding (Munyiri *et al.*, 2015; Rebe *et al.*, 2004).

Chemical control of stem borers

Insecticides

Since the early 1950s chemical insecticides were an important component of insect pest control (Dent, 2000). However new approaches are incorporated in IPM systems today, although insecticides are still used as a corner stone in pest management (Dent, 2000).

Over the past few decades, chemical control has been the most important strategy to control stem borers in South Africa (Van den Berg *et al.*, 1994). Bate and Van Rensburg (1992) reported that 40% of maize plants that have whorl damage at 2 weeks after infestation is the level at which action must be taken to control *C. partellus*. Studies by Slabbert and Van den Berg (2009) showed that successful penetration of insecticides into the plant whorl was critically important for *C. partellus* control. It should but be kept in mind that the overall percentage of *C. partellus* larvae that occur behind the leaf sheaths of maize plants are only 20% and that even the most effective whorl applications will not reach these larvae (Slabbert & Van den Berg, 2009).

Bt maize

Bacillus thuringiensis (Bt) was first discovered in the early 1900's in larvae of silkworms and meal moth that were diseased. Only 20 years after this, its potential for pest control were recognized (Heckel, 2012). The spores of Bt can be isolated from different resources in the environment such as soil, fresh water, grain dusts or other animals such as annelids, crustaceans and insectivorous mammals (Raymond *et al.*, 2010). This gram-positive bacterium produces proteinaceous crystals known as δ -endotoxins and these crystals, coded for by specific genetic sequences are toxic to a several species of insects (Douville *et al.*, 2005). The Bt gene that encodes for these protein sequences is isolated from the *B. thuringiensis* bacterium and inserted into the plant's genome sequence so the plant may produce the insecticidal protein toxins making it resistant to insect attacks (Lu *et al.*, 2007).

For the toxin to be effective, the Cry proteins need to be solubilised in their crystal form in the insect midgut. These crystals consist of protoxins and become activated when a susceptible insects eats it and the insect midgut proteases process it (Schnepf *et al.*, 1998). The Cry protoxin is then digested to a small toxin protein, thus activating the Cry toxin. Cry toxins that

are activated have two functions namely receptor binding and ion channel activity (Schnepf *et al.*, 1998). This toxin that has been activated binds to a protein receptor on the columnar cell of the midgut of the insect (Gill, 1995). After the binding occurs into the cell membrane, the toxin must change its appearance. These toxins then form pores that are permeable to any small ion and molecules, thus disrupting the osmotic balance. The disrupted ion regulation causes loss in function and potentially the midgut later on (Gill, 2005). This causes cell lysis and ends in death for the insect.

The initial Bt maize was developed for the control of the two stem borers *Ostrinia nubilalis* (Hübner) and *Diatraea grandiosella* (Dyar) in North America (Archer *et al.*, 2001). Genetically modified Cry1Ab maize for lepidopteran pest control was first commercialized in South Africa in 1997 and after a few years later during the growing season of 2012/13, stacked gene hybrids that express both the Cry1A.105 and Cry2Ab2 toxins were planted (Gouse *et al.*, 2005; Van den Berg *et al.*, 2013).

1.6 Aim and objectives

The aim of the study was to determine the response of *Chilo partellus* larvae and moths to Bt and non-Bt maize in different cropping scenarios.

The specific objectives were to:

- determine if *C. partellus* larvae distinguish between non-Bt and Bt maize plants and how this affects their behaviour
- determine if *C. partellus* moths distinguish between non-Bt and Bt maize plants in their oviposition choices
- screen *C. partellus* larvae from different regions of South Africa for resistance to Bt maize events
- determine the effect of larval age on their survival on Bt maize

1.7 References

Abrol, D.P. & Shankar, U. 2012. Integrated pest management: principles and practices. Cambridge, USA: CABI. p. 1-2.

Ajala, S.O. & Saxena, K.N. 1994. Interrelationship among *Chilo partellus* (Swinhoe) damage parameters and their contribution to grain yield reduction in maize (*Zea mays* L.). *Applied Entomology and Zoology*, 29: 460-476.

Ampong-Nyarko, K., Seshu Reddy, K.V, Nyang'or, R.A.& Saxena, K.N. 1994. Reduction of pest attack on sorghum and cowpea by intercropping. *Entomologia Experimentalis et Applicata*, 70:179–84.

Archer, T.L., Patrick, C., Schuster, G., Cronholm, G., Bynum Jr, E.D. & Morrison, W.P. 2001. Ear and shank damage by corn borers and corn earworms to the four events of *Bacillus thuringiensis* transgenic maize. *Crop Protection*, 20:139-144.

Bate, R. & Van Rensburg, J.B.J. 1992. Predictive estimation of maize yield loss caused by *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in maize. *South African Journal of Plant and Soil*, 9:150-154.

Bayram, A. & Tonga, A. 2015. First report of *Chilo partellus* in Turkey, a new invasive maize pest of Europe. *Journal of Applied Entomology*, 1-5.

Belay, D. & Foster, J.E. 2010. Efficacies of habitat management techniques in managing maize stem borers in Ethiopia. *Crop Protection*, 29: 422-428.

Ben-Yakir, D., Chen, M., Sinev, S. & Seplyarsky, V. 2013. *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) a new invasive species in Israel. *Journal of Applied Entomology*, 137: 398-400.

Berger, A. 1989. Ballooning activity of *Chilo partellus* larvae in relation to size of moths egg batches, eggs and larvae and age of mother. *Entomologia Experimentalis et Applicata*, 50: 125-132.

Berger, A. 1989. Egg weight, batch size and fecundity of the spotted stalk borer, *Chilo partellus* in relation to weight of females and time of oviposition. *Entomologia Experimentalis et Applicata*, 50: 199-207.

Berger, A. 1992. Larval movements of *Chilo partellus* (Lepidoptera: Pyralidae) within and between plants: timing, density responses on survival. *Bulletin of Entomological Research*, 82: 441-448.

- Bleszynski, S. 1970. A revision of the world species of *Chilo* Zincken (Lepidoptera: Pyralidae). *Bulletin of the British Museum (Natural History), Entomology*, 25: 101-195.
- Brankatsck, G. & Finkbeiner, M. 2015. Modelling crop rotation in agricultural LCA's- challenges and potential solutions. *Agricultural Systems*, 138: 66-76.
- Bravo, A., Gill, S.S. & Soberón, M. 2006. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon*, 49: 432-435
- Can Cengiz, F., Kaya, K., Ulasli, B. & Morinière, J. 2016. First record of the egg parasitoid of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in Turkey using DNA barcoding. *Turkish Journal of Entomology*, 40:125-131.
- Cherry, A.J. Lomer, C.J. Djegui, D. & Schulthess, F. 1999. Pathogen incidence and their potential as microbial control agents in IPM of maize stem borers in West Africa. *Biocontrol*, 44: 301-327.
- Chu, C., Spencer, J.L., Curzi, M.J., Zavala, J.A. & Seufferheld, M.J. 2013. Gut bacteria facilitates adaptation to crop rotation in the western corn rootworm. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 11917-11922.
- Crickmore, D., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. & Dean D.H. 1998. Revision of the Nomenclature for the *Bacillus thuringiensis* Pesticidal Crystal Proteins. *Microbiology and Molecular Biology Reviews*, 62: 807-813.
- Deep, D.S. & Rose, H.S. 2014. Study on the external morphology of the eggs of maize borer *Chilo partellus* (Swinhoe). *Journal of Entomology and Zoology Studies*, 2:187-189.
- Dent, D. 1991. Insect pest management. CAB International. Wallingford, UK.
- Dent, D. 2000. Insect pest management. 2nd ed. CAB International. Wallingford, UK.
- Douville, M., Gagné, F., Masson, L., McKay, J. & Blaise, C. 2005. Tracking the source of *Bacillus thuringiensis* Cry1Ab in the environment. *Biochemical Systematics and Ecology*, 33: 219-232.
- Ehler, L.E. 2006. Perspectives integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest Management Science*, 62: 787-789.

FAO. 2017. Data. <http://www.fao.org/faostat/en/#data/QC>. Date of access: 31 January 2017.

FAS/USDA. 2017. United States Department of Agriculture and Foreign Agricultural Service. WorldagriculturalproductionSeriesWAP01-17.www.usda.mannlib.cornell.edu/usda/fas/worldag-production//2010s/2017/worldagproduction-01-12-2017.pdf Date of access: 31 January 2017.

Gilbert, L.I. 2009. Insect development: morphogenesis, molting and metamorphosis. Academic Press: London.

Gill, S. 1995. Mechanism of action of *Bacillus thuringiensis* toxins. *Memoirs of the Institute of Oswaldo Cruz*, 9: 69-74.

.

Gouse, M., Pray, C.E., Kirsten, J. & Schimmelpfennig, D. 2005. A GM subsistence crop in Africa: the case of Bt white maize in South Africa. *International Journal of Biotechnology*, 7: 84-94.

Hari, N.S., Jindal, J., Malhi, N.S. & Khosa, J.K. 2008. Effect of adult nutrition and density on the performance of spotted stem borer, *Chilo partellus* in laboratory cultures. *Journal of Pest Science*, 81: 23-27.

Heckel, D.G. 2012. Learning the ABC's of Bt: ABC transporters and insect resistance to *Bacillus thuringiensis* provide clues to a crucial step in toxin mode of action. *Pesticide Biochemistry and Physiology*, 104:103-110.

Hutchinson, W.D., Venette, R.C., Bergvinson, D. & Van den Berg, J. 2008. Pest distribution profile: *Chilo partellus*. Developed in cooperation with the Harvest Choice Workshop, CIMMYT. Harvest Choice.

James, C. 2015. Global status of commercialized biotech/GM crops: 2015. *ISAAA Brief No.51*. ISAAA: Ithaca, NY.

Jurat-Fuentes, J.L. & Crickmore, N. 2016. Specificity determinants for Cry insecticidal proteins: insights from their mode of action. *Journal of Invertebrate Pathology*, <http://dx.doi.org/10.1016/j.jip.2016.07.018>.

Kalaisekar, A., Padmaja, P. G., Bhagwat, V. R. & Patil, J. V. 2017. Insect pests of millets: systematics, bionomics, and management. London, United Kingdom, Elsevier/Academic Press. <http://public.ebib.com/choice/publicfullrecord.aspx?p=4773782>.

Kfir, R. 1990. Parasites of the spotted stalk borer, *Chilo partellus* (Lepidoptera: Pyralidae) in South Africa. *Entomophaga*, 35: 403-410.

Kfir, R. 1991. Effect of diapause on development and reproduction of the stem borers *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, 84:1677-1680.

Kfir, R. 1993. Diapause termination in the spotted stem borer *Chilo partellus* (Lepidoptera: Pyralidae) in the laboratory. *Annals of Applied Biology*, 123:1-7.

Kfir, R. 1994. Attempts at biological control of the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in South Africa. *African Entomology*, 2: 67-68.

Kfir, R., Overholt, W.A., Khan, Z.R. & Polaszek, A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology*, 47: 701-731.

Khan, Z.R., Midega, C.A.O., Hutter, N.J., Wilkins, R.M. & Wadhams, L.J. 2006. Assessment of the potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for management of *Chilo partellus*. *Entomologia Experimentalis et Applicata*, 119:15-22.

Kogan, M. 1998. Integrated pest management: historical perspectives and contemporary development. *Annual Review of Entomology*, 43: 243-270.

Kumar, H. & Saxena, K.N. 1992. Resistance in certain maize cultivars to first and third instar *Chilo partellus* larvae. *Entomologia Experimentalis et Applicata*, 65:75-80.

Kumar, H. & Saxena, K.N. 1994. Infestation and damage on three maize cultivars by the stalk borer *Chilo partellus* (Swinhoe) in relation to their yield in western Kenya. *Insect Science and its Application*, 15: 331-335.

Kumar, H. 1997. Resistance in maize to *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): an overview. *Crop Protection*, 16: 243-250.

Lu, G., Hu. & Bidney, D.L. 2007. Sunflower. (In: Nagata, T., Lörz, H. & Widholm, J.M., ed. *Biotechnology in agriculture and forestry*. Berlin: Heidelberg. 39-58.)

Maluleke, M.H., Addo-Bediako, A. & Ayisi, K.K. 2005. Influence of maize/ lablab intercropping on lepidopterous stem borers infestation in maize. *Journal of Economic Entomology*, 98: 384-388.

Mashwani, M.A., Ullah, F., Ahmad, S., Sohail, K., Shah, S.F. & Usman, M. 2015. Infestation of maize stemborer, *Chilo partellus* (Swinhoe) in maize stubbles and stalks. *Journal of Biodiversity and Environmental Sciences*, 7:180-185.

Mgoo, V.H., Makundi, R.H., Pallangyo, B., Schulthess, F., Jiang, N. & Omwega, C.O. 2006. Yield loss due to the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) at different nitrogen application rates to maize. *Annales de la Société Entomologique de France*, 42487-494.

Mikac, K.M., Douglas, J. & Spencer, J.L. 2013. Wing shape and size of the western corn rootworm (Coleoptera: Chrysomelidae) is related to sex and resistance to soybean-maize crop rotation. *Journal of Economic Entomology*, 106: 1517-1524.

Munyiri, S.W., Mugo, S.N. & Mwololo, J.K. 2015. Mechanisms and levels of resistance in hybrids, open pollinated varieties and landraces to *Chilo partellus* maize stem borers. *International Research Journal of Agricultural Science and Soil Science*, 5: 81-90.

Mutisya, D.L., Tefera, T. & Mugo, S. 2013. Quality of field collected and laboratory reared *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) for screening maize genotypes. *African Journal of Biotechnology*, 12: 5370-5374.

Neupane, S., Bhandari, G., Sharma, S.D., Yadav, S. & Subedi, S. 2016. Management of stem borer (*Chilo partellus* Swinhoe) in maize using conventional pesticides in Chitnan, Nepal. *Journal of Maize Research and Development*, 2:13-19.

Ntiri, E.S., Calatayud, P.A., Van den Berg, J., Schulthess, F. & Le Ru, B.P. 2016. Influence of temperature on intra- and interspecific resource utilization within a community of lepidopteran maize stemborers. *PLoS ONE* 11(2):e0148735. Doi:10.1371/journal.pone.0148735.

Obonyo, D.N., Songa, J.M., Oyieke, F.A., Nyamasyo, G.H.N. & Mugo, S.N. 2008. Bt- transgenic maize does not deter oviposition by two important African cereal stemborers, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Journal of Applied Biosciences*, 10: 424-433.

Ofomata, V.C., Overholt, W.A., Lux, S.A., Van Huis, A. & Egwuatu, E.G. 2000. Comparative studies on the fecundity, egg survival, larval feeding and development of *Chilo partellus* and *Chilo orichalcociliellus* (Lepidoptera: Crambidae) of five grasses. *Annals of the Entomological Society of America*, 93: 492-499.

Panchal, B.M. & Kachole, M.S. 2013. Life cycle of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) on an artificial diets. *International Journal of Plant, Animal and Environmental Sciences*, 3: 19-22.

Pedda Kasim, D., Suneetha, P., Srideepthi, R., Lakshmi Sahithya, U. & Krishna, M.S.R. 2016. Survival and development of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) green gram based diet in laboratory conditions. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7: 561-567.

Pimental, D. & Peshin, R. 2014. Integrated pest management: pesticide problems. Vol 3. Springer: New York. p. 7-8.

Prinsloo, G.L. & Uys, V.M. 2015. Insects of cultivated plants and natural pastures in Southern Africa. *Entomological Society of Southern Africa*. p. 104-132.

Raymond, B., Johnston, P.R., Nielsen-LeRoux, C., Lereclus, D. & Crickmore, N. 2010. *Bacillus thuringiensis*: An impotent pathogen? *Trends in Microbiology*, 18:189-194.

Razig, A.A.M.A & Ishag, J. 2014. Population dynamics of spotted stem borer *Chilo partellus* (Swinhoe) on some maize *Zea mays* (L.) varieties at Rahad area, Sudan. *Persian Gulf Crop Protection*, 3:6-10.

Rebe, M., Van den Berg, J. & Donaldson, G. 2004. The status of leaf feeding resistance and oviposition preference of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) for sweet sorghum (*Sorghum bicolor*) landraces. *International Journal of Pest Management*, 50: 49-53.

SAGIS. 2017. Monthly announcement of data- Maize. SMD-01-2017. http://www.sagis.org.za/smd_20170125b.html. Date of access: 31 January 2017.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. & Dean, D.H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews*, 62:775-806.

Seshu Reddy, K.V. & Sum, K.O.S. 1991. Determination of economic injury level of the stem borer, *Chilo partellus* (Swinhoe) in maize, *Zea mays* L. *International Journal of Tropical Insect Science*, 12: 269-274.

Shelton, A.M., Zhao, J.Z. & Roush, R.T. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology*, 47: 845-881.

Shelton, A.M. & Badenes-Perez, F.R. 2006. Concepts and applications of trap cropping in pest management. *Annual Review of Entomology*, 51: 285-308.

Siddalingappa, C.T., Venkatesh, H. & Shivasharanappa, Y. 2010. Biology of maize stem borer, *Chilo partellus* (Swinhoe) Crambidae: Lepidoptera. *International Journal of Plant Protection*, 3: 91-93.

Singh, B.U., Sharma, H.C. & Rao, K.V. 2012. Mechanisms and genetic diversity for host plant resistance to spotted stem borer, *Chilo partellus*, in sorghum, *Sorghum bicolor*. *Journal of Applied Entomology*, 136: 386-400.

Sithole, S.Z. 1990. Status and control of the stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Southern Africa. *Insect Science and its Application*, 11: 481-485.

Skoroszewski, R.W. & Van Hamburg, H. 1987. The release of *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) against stalk-borers of maize and grain-sorghum in South Africa. *Journal of Entomological Society of southern Africa*, 50: 249-255.

Slabbert, O. & Van den Berg, J. 2009. The effect of the adjuvant, Break-Thru S240 on whorl penetration and efficacy of foliar insecticide applications against *Chilo partellus*. *South African Journal of Plant Soil*, 26: 254-257.

Skovgård, H. & Päts, P. 1997. Reduction of stemborer damage by intercropping maize with cowpea. *Agriculture, Ecosystems and Environment*, 62:13-19.

Smart, G.C. 1995. Entomopathogenic nematodes for the biological control of insects. *Journal of Nematology*, 27:529-534.

Stern, V.M., Smith, R.F., van den Bosch, R. & Hagen, K.S. 1959. The integrated control concept. *Hilgardia*, 29:81-101.

Swinhoe, C. 1885. On the Lepidoptera of Bombay and the Deccan. Heterocera. *Proceedings of the Zoological Society of London* 1885, 2: 287–307.

Sylvain, N.M., Manyangarirwa, W., Tuarira, M. & Onesime, M.K. 2015. Effect of the Lepidoptera stem borers, *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) on green mealies production. *International Journal of Innovative Research and Development*, 4:366-374.

Tamiru, A., Bruce, T.J.A., Woodcock, C.M., Caulfield, J.C., Midega, C.A.O., Ogot, C.K.P.O., Mayon, P., Birkett, M.A., Pickett, J.A. and Khan, Z.R. 2011. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology Letters*, doi:10.1111/j.1461-0248.2011.01674.x

Tende, R.M., Nderitu, J.H., Mugo, S., Songa., Olubay, F. & Bergvinson, D. 2005. Screening for development of resistance by the spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) to Bt maize delta-endotoxins. *African Crop Science Conference Proceedings*, 7:1241-1244.

Van den Berg, J. 2006. Vetiver grass (*Vetivera zizanioides* (L.) Nash) as trap plant for *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France* (N.S.), 42:449-454.

Van den Berg, J., Hilbeck, A. & Bøhn, T. 2013. Pest resistance to Cry1Ab Bt maize: field resistance, contributing factors and lessons from South Africa. *Crop Protection*, 54:154-160.

Van den Berg, J. & Van Hamburg, H. 2015. Trap cropping with Napier grass, *Pennisetum purpureum* (Schumach), decrease damage by maize stem borers. *International Journal of Pest Management*, 61:73-79.

Van den Berg, J. & Van Rensburg, J.B.J. 1991. Infestation and injury levels of stem borers in relation to yield potential of grain sorghum. *South African Journal of Plant Soil*, 8:127-131.

Van den Berg, J., Van Rensburg, J.B.J. & Pringle, K.L., 1991. Comparative injuriousness of *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae) on grain sorghum. *Bulletin of Entomological Research*, 82: 137- 143.

Van den Berg, J., Van Rensburg, G.D.J. & Van der Westhuizen, M.C. 1994. Host plant resistance and chemical control of *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller) in an integrated pest management system on grain sorghum. *Crop Protection*, 13: 307-310.

Van Hamburg, H. 1971. Die bio-ekologie van die kafferkoringstamruspe. Pretoria: UP (Verhandeling-MSc).

Van Hamburg, H. 1976. Die bionomie en ekonomiese belangrikheid van die graansorghumstamruspe, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Unpublished MSc thesis, University of Pretoria.

Van Hamburg, H. 1979. The grain-sorghum stalk-borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): seasonal changes in adult populations in grain sorghum in the Transvaal. *Journal of Entomological Society of Southern Africa*, 42:1-9.

Van Hamburg, H. & Hassell, M.P. 1984. Density dependence and the augmentative release of egg parasitoids against graminaceous stalkborers. *Ecological Entomology*, 9:101-108.

Wahedi, J.A., David, D.L., Danba, E.P., Yisa, S. & Zakariya, R. 2016. Yield performance of maize treated with neem seed extracts against stem borers. *American Journal of Experimental Agriculture*, 12:1-8.

Yonow, T., Kriticos, D.J., Ota, N., Van den Berg, J. & Hutchinson, W.D. 2016. The potential global distribution of *Chilo partellus*, including consideration of irrigation and cropping patterns. *Journal of Pest Science*, doi:10-1007/s10340-016-0801-4.

CHAPTER 2

THE STATUS OF RESISTANCE OF *CHILO PARTELLUS* TO BT MAIZE IN SOUTH AFRICA

2.1 Abstract

Chilo partellus is one of the main lepidopteran pests of maize in South Africa and has been effectively controlled by Bt maize since its cultivation commenced during 1998. The non-compliance to planting non-Bt maize refuges as part of an insect resistance management strategy contributed to resistance evolution of another stem borer species, *Busseola fusca*, in South Africa. To improve compliance to refuge requirements, a different strategy to the high-dose/refuge strategy approach, namely “refuge in a bag” has been suggested as an alternative. This strategy implies the mixture of Bt and non-Bt seed in a field in order to provide a random refuge and to address the issue of non-compliance by farmers. The aim of this study was to determine the susceptibility of *C. partellus* to Bt maize and to determine if older and larger *C. partellus* larvae could survive on Bt maize. The scenarios in this study therefore mimicked cases in which larval migration takes place from non-Bt plants to Bt plants inside a seed mixture planting. Laboratory studies were conducted in which larvae of four populations of *C. partellus* were reared on whorl tissue of two different Bt maize events and their non-Bt iso-hybrid. To evaluate the effect of larval size on susceptibility to Bt toxin, neonate larvae were put onto Bt maize whorl tissue or reared on non-Bt maize for 7 or 16 days before they were transferred to Bt maize tissue. Larval survival and mass was recorded and compared to survival and development on non-Bt maize tissue, which served as control treatment. Results indicated that *C. partellus* is highly susceptible to Bt maize in South Africa, even after nearly 20 years of continued exposure to selection pressure for resistance evolution to Bt toxin. Results further showed that the older the larvae, the higher the likelihood of survival on Bt maize. It can be concluded that migratory behavior of larvae may lead to exposure to sub-lethal exposure to Cry protein concentrations in Bt maize plants, thereby contributing to resistance evolution in a seed mixture scenario.

Keywords: Bt maize, *Chilo partellus*, insect resistance management

2.2 Introduction

The initial development of Bt maize was for the control of the two stem borer species, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae), in North America (Archer *et al.*, 2001). Cultivation of Bt maize in South Africa commenced during 1998 and targeted *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Gouse *et al.*, 2005). The planting of Bt crops results in a high selection pressure for resistance evolution which may lead to a rapid evolution of resistance in target pests (Yang *et al.*, 2014). Van Rensburg (2007) reported that *B. fusca* developed field resistance to the single gene (Cry1Ab) Bt maize, eight years after its first planting in South Africa. No reports of either field resistance or survival of *C. partellus* on Bt maize has however been reported in South Africa. No monitoring of the resistance status of *C. partellus* has been done in South Africa and results on its susceptibility to Bt maize are limited to those of Van Rensburg (1999) which showed that this pest was highly susceptible to Cry1Ab expressing maize.

According to Van den Berg *et al.* (2013) field evolved resistance is when a pest population is no longer susceptible to a toxin and when pest individuals have the ability to complete their life cycle on Bt maize under field conditions. Insect resistance management (IRM) strategies have been developed to delay resistance evolution and involve implementation of the high-dose/refuge strategy or seed mixture strategies. According to Yang *et al.* (2014) the high-dose/refuge concept implies that the Bt-susceptible insects that are produced in the refuge area mate with the moths from the rare resistant types that survive in the Bt crop, to create offspring that are heterozygous and therefore susceptible to Bt maize.

Seed mixture strategies requires little effort from growers to implement resistance management since it involves the planting of Bt and non-Bt seed mixtures in the same field (Carroll *et al.*, 2012). This strategy reduces the probability of Bt-resistant adults to mate with each other and thus favours randomised mating between susceptible and resistant insects (Carroll *et al.*, 2012). However, this strategy has its limits because larval movement between Bt and non-Bt plants might accelerate the evolution of pest resistance in seed mixtures (Carroll *et al.*, 2012). Heterozygote fitness and selection pressure in seed mixture plantings could be increased since larger migrating larvae do not receive a lethal dose of the toxin when they feed on non-Bt plants and then migrate to Bt plants (Carroll *et al.*, 2012). If susceptible larvae migrate from non-Bt

plants to Bt plants the efficiency of the refuge is reduced, because fewer susceptible larvae will be left on non-Bt plants (Carroll *et al.*, 2012).

The objectives of the study were to determine *C. partellus* resistance status to Bt maize and to determine the effect of larval age on its ability to survive on Bt maize in a scenario where migration may occur between non-Bt and Bt plants in seed mixture plantings.

2.3 Materials and methods

A laboratory study was conducted at the Agricultural Research Council - Grain Crops Institute (ARC-GCI) in Potchefstroom. Four populations of *C. partellus* were collected from Prieska, Vaalharts, Vrededorst and Potchefstroom respectively. Larvae were reared in insect rearing chambers maintained at $27\pm 2^{\circ}\text{C}$, 40 to 70% relative humidity (RH) under a 14:10h light:dark (L:D) photoperiod.

Two Bt maize events, the single gene (Cry1Ab) event (referred to as Bt1 in this study) and stacked gene event (Cry1A.105 + Cry2Ab2) (referred to as Bt2 in this study) were used as well as their non-Bt iso-hybrid. Three laboratory experiments were conducted in which larvae were reared on maize whorl tissue that was cut from maize plants grown under field conditions. In these experiments, treatments were designed to simulate different migration scenarios in which larvae of different ages (first instar, 7 and 16-day old) move from non-Bt to Bt maize plants.

2.3.1. Laboratory bioassay

First instar larvae

Neonate larvae were inoculated onto maize whorl tissue of the non-Bt, Bt1 and Bt2 treatments. Five larvae were put onto freshly cut whorl tissue inside plastic containers (100 ml) (Fig. 2.1). Each treatment was replicated three times. Each replicate consisted of ten containers with five larvae per container. Larval survival and mass were determined 7, 10, 14, 17 and 21 days after commencement of feeding. Fresh whorl tissue was provided at each sampling interval.



Figure 2-1: First instar larvae inoculated onto maize whorl tissue in a 100 ml plastic container.

Seven-day old larvae

In order to get larvae of the appropriate age, neonates were inoculated onto non-Bt maize whorl tissue and allowed to feed for 7 days. Larvae were then transferred to other containers onto the leaf tissue as follows: non-Bt to Bt1, non-Bt to Bt2 and non-Bt to non-Bt (control treatment). Larval survival and mass were determined at 7 and 14 days after transfer and fresh food was provided as described above. This experiment was replicated three times with five larvae per container (8-10 containers per replicate depending on larval survival before the first transfer).

Sixteen-day old larvae

In order to get larvae of the appropriate age, neonates were inoculated onto non-Bt maize whorl tissue and allowed to feed for 16 days. Larvae were then transferred from the non-Bt maize tissue as follows: non-Bt to Bt1, non-Bt to Bt2 and non-Bt to non-Bt (control treatment). Larval survival and mass were determined 5, 10 and 15 days after transfer and fresh whorl tissue provided at each interval. This experiment was replicated three times with five larvae per container (6-9 containers per replicate depending on larval survival before first transfer). Pupal mass, sex ratio and percentage pupation was determined at the end of the experiment.

Data analysis

Data were analysed using Gensstat edition 16. Larval survival and mass was analysed by means of one-way ANOVA. Pupation percentage and sex ratios were analysed by means of

Chi-square analysis. Pupal mass was analysed by means of a Student t-test. Significant differences were determined with Tukey test if $P < 0.05$. For the 16-day old larvae, corrected percentage mortality was used, using Abbott's equation (Abbott, 1925) and the significant differences between treatments for corrected percentage mortality was analysed by means of Student t-tests.

2.4 Results

2.4.1 Evaluation of larval survival and growth

First instar larvae

Significant differences ($p < 0.05$) were observed between larval survival on non-Bt and both Bt treatments for all four populations (Figs. 2-2 to 2-5). However, no significant differences were observed between the survival of larvae on the two Bt treatments. Larval survival of all populations decreased rapidly over the first 7 days on the two Bt treatments (Figs. 2-2 to 2-5). The Bt treatments effectively controlled the first-instar larvae and no survival was observed on day 7 for any of the four populations on the two Bt treatments. Larval survival on the non-Bt control treatments ranged between 42.7% and 58.7% for the four populations on day 21. On non-Bt maize, larval mass increased over the 21 days for all populations.

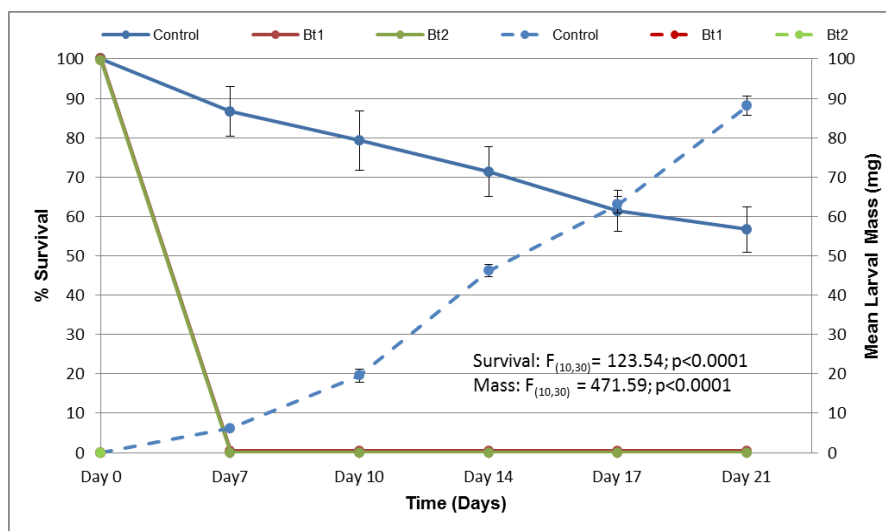


Figure 2-2: The mean percentage survival and mean mass over 21 days for first instar *Chilo partellus* larvae of the Potchefstroom population (Bars = Standard Error (SE)). Solid lines indicate larval survival whereas dotted lines indicate mean larval mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

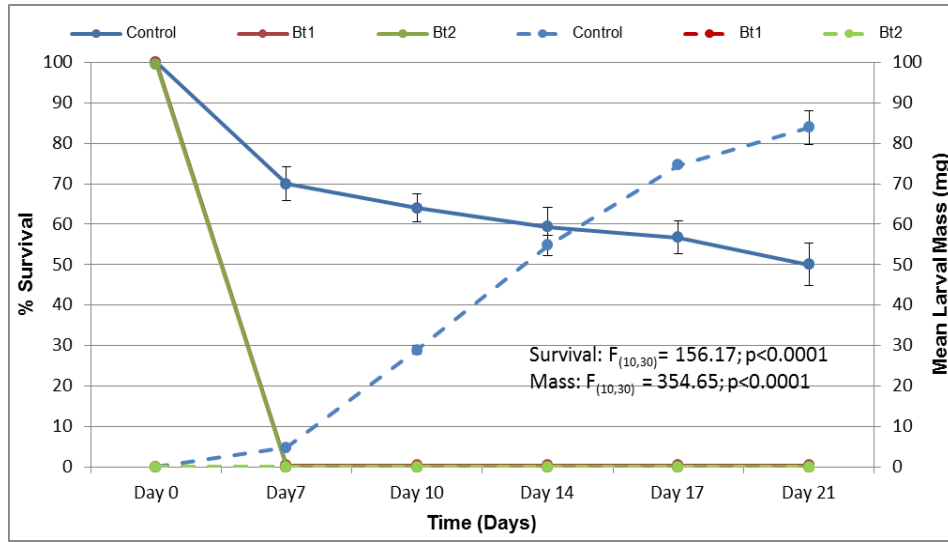


Figure 2-3: The mean percentage survival and mean mass over 21 days for first instar *Chilo partellus* larvae of the Prieska population (Bars = SE). Solid lines indicate larval survival whereas dotted lines indicate mean larval mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

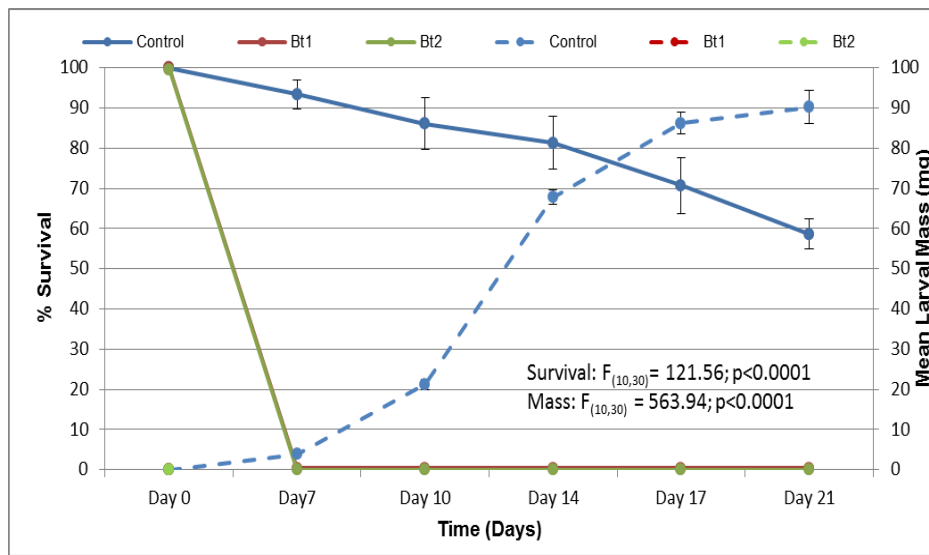


Figure 2-4: The mean percentage survival and mean mass over 21 days for first instar *Chilo partellus* larvae of the Vaalharts population (Bars = SE). Solid lines indicate larval survival whereas dotted lines indicate mean larval mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

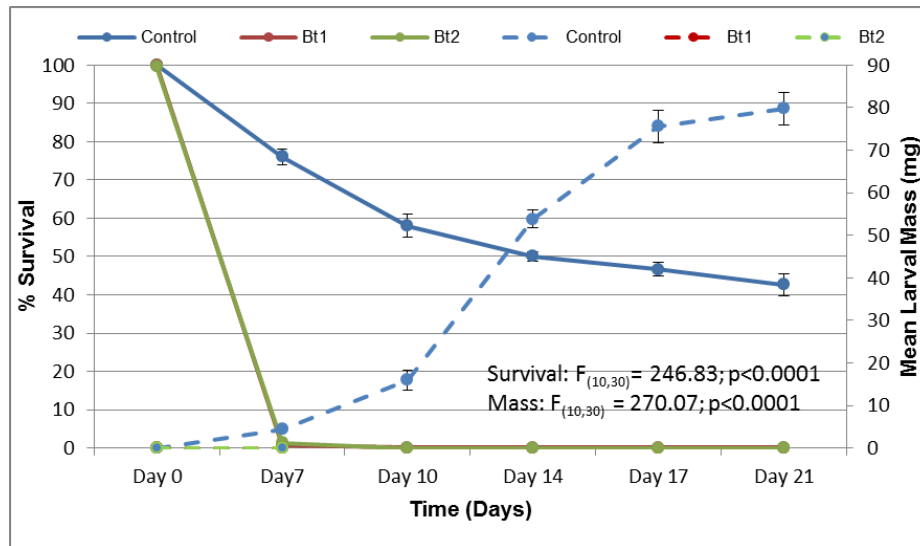


Figure 2-5: The mean percentage survival and mean mass over 21 days for first instar *Chilo partellus* larvae of the Vredefort population (Bars = SE). Solid lines indicate larval survival whereas dotted lines indicate larval mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

Seven-day old larvae

A significant difference ($p < 0.05$) was observed between larval survival on non-Bt and both Bt treatments for all populations after transferred larvae were allowed to feed for a week or more on Bt maize (Figs. 2-6 & 2-7). The differences between the two Bt maize treatments were however not significant. None of the 7-day old larvae of the Potchefstroom and Prieska populations survived for longer than 7 days after transfer to the two Bt treatments (Figs. 2-6 & 2-7). However, 0.66% and 2.33% of the Vaalharts population (Fig. 2-8) survived on whorl tissue of Bt1 and Bt2, respectively. Larvae of the Vredefort population (Fig. 2-9) showed survival of 7.71% on Bt1 and 2.34% on Bt2, 7 days after transfer to the respective treatments. No survival of any of the 7-day old larvae was observed after an additional 14 days of feeding on the respective Bt maize treatments.

A rapid decrease in larval mass was observed over 7 days. Surviving larvae of the Vaalharts population had a mean larval mass of 0.09 and 0.27 mg.larva⁻¹ for Bt1 and Bt2, respectively while that of larvae that developed on the non-Bt tissue was 47.17 mg (Fig. 2-8). Larvae of the Vredefort population had a mean larval mass of 0.46 and 0.11 mg.larva⁻¹ for Bt1 and Bt2 respectively on day 7 (Fig. 2-9). Larval mass increased for all *C. partellus* populations on the non-Bt treatment.

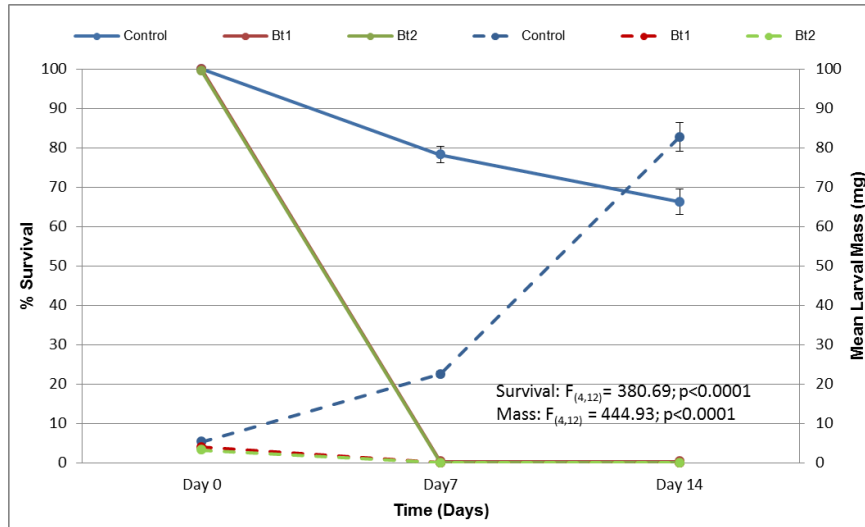


Figure 2-6: The mean percentage survival and mean mass of seven-day old *Chilo partellus* larvae over a 14-day period on Bt maize after transfer from non-Bt maize (Potchefstroom population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

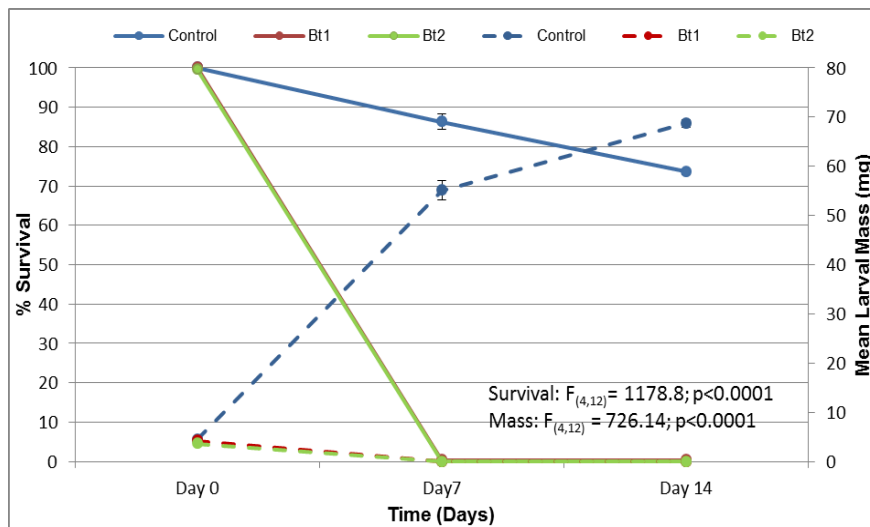


Figure 2-7: The mean percentage survival and mean mass of seven-day old *Chilo partellus* larvae over a 14-day period on Bt maize after transfer from non-Bt maize (Prieska population) (Bars = SE). Solid lines indicate the survival whereas dotted lines indicate the mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

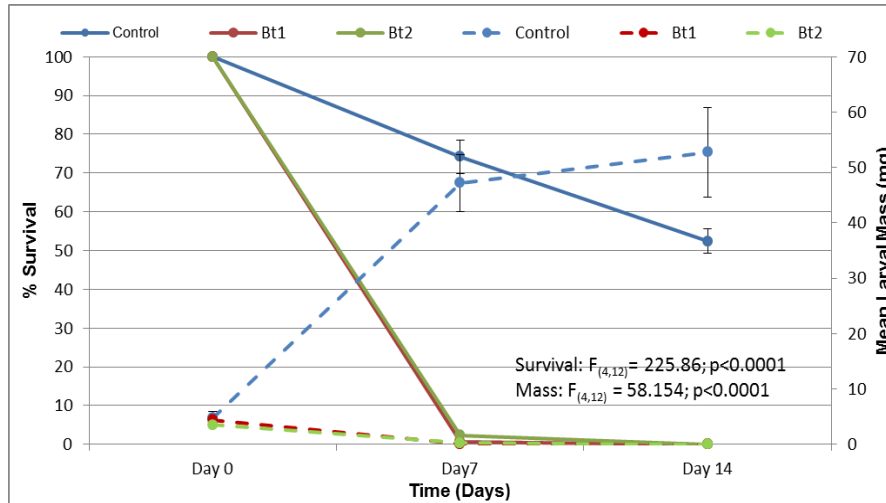


Figure 2-8: The mean percentage survival and mean mass of seven-day old *Chilo partellus* larvae over a 14-day period on Bt maize after transfer from non-Bt maize. (Vaalharts population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

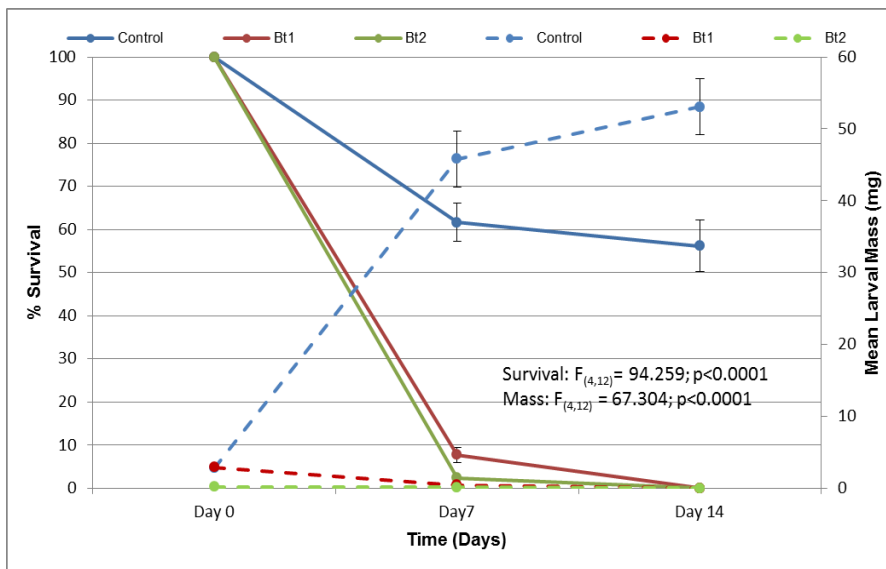


Figure 2-9: The mean percentage survival and mean mass of seven-day old *Chilo partellus* larvae over a 14-day period on Bt maize after transfer from non-Bt maize. (Vredefort population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

Sixteen-day old larvae

Significantly lower numbers of larval survival of all four populations between non-Bt and both Bt treatments were observed for larvae that were transferred as 16-day old larvae from non-Bt to Bt maize (Figs. 2-10, 2-12, 2-13). However, no significant differences were observed between the percentage surviving larvae on the two Bt treatments. Larval survival for the Potchefstroom, Vaalharts and Vredefort populations ranged between 0 and 14% on the two Bt treatments on

day 15 (Figs. 2-10, 2-12, 2-13). Larvae of the Potchefstroom population had the lowest survival on Bt1 (0%) and Bt2 (2.5%), while larvae of the Vaalharts and Vredefort populations had a higher survival on Bt1 and Bt2 than the Potchefstroom population. The Prieska population had the highest percentage larval survival on the Bt treatments with, 33% on Bt1 and 28% on Bt2 on day 15 (Fig. 2-11).

Mean larval mass of all four populations were significantly higher on non-Bt maize compared to Bt1 and Bt2. Mean larval mass of each population on Bt maize remained virtually the same over time until the experiment was terminated 15 days after commencement of feeding on Bt maize tissue (Figs. 2-10 to 2-13). Although mean larval mass of the Potchefstroom population on Bt2 maize remained virtually the same over time it was significantly higher than that of larvae feeding on Bt1 after 15 days (Fig. 2-10.)

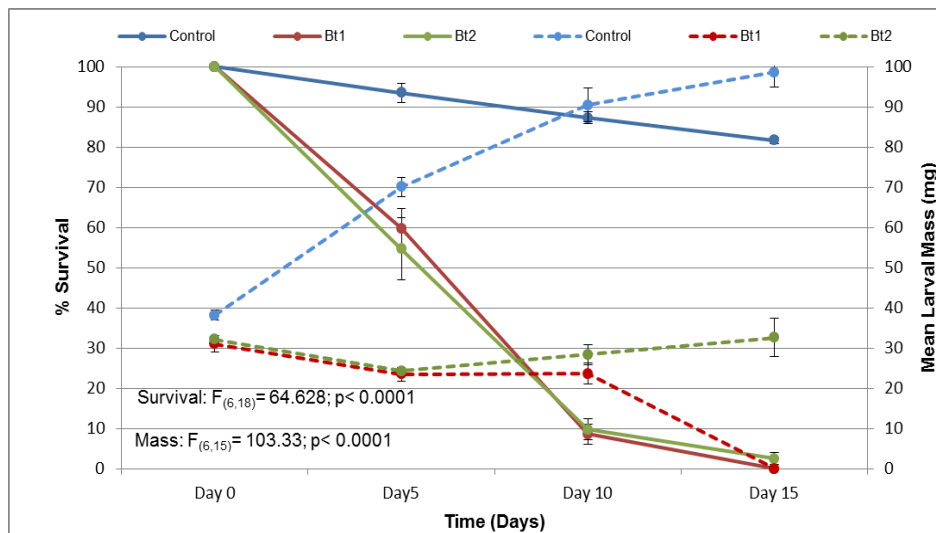


Figure 2-10: The mean percentage survival and mean mass of sixteen- day old *Chilo partellus* larvae over a 15-day period on Bt maize after transfer from non-Bt maize. (Potchefstroom population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

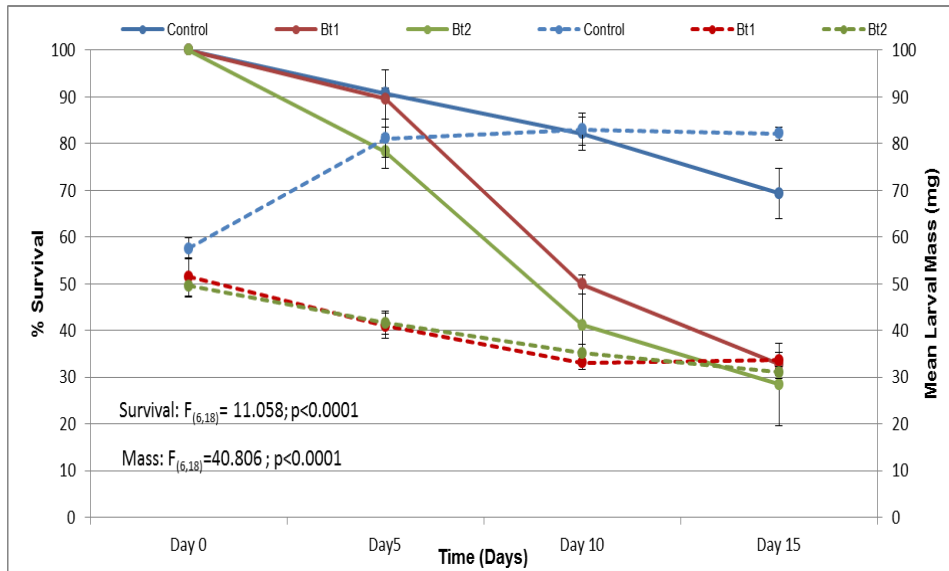


Figure 2-11: The mean percentage survival and mean mass of sixteen-day old *Chilo partellus* larvae over a 15-day period on Bt maize after transfer from non-Bt maize. (Prieska population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

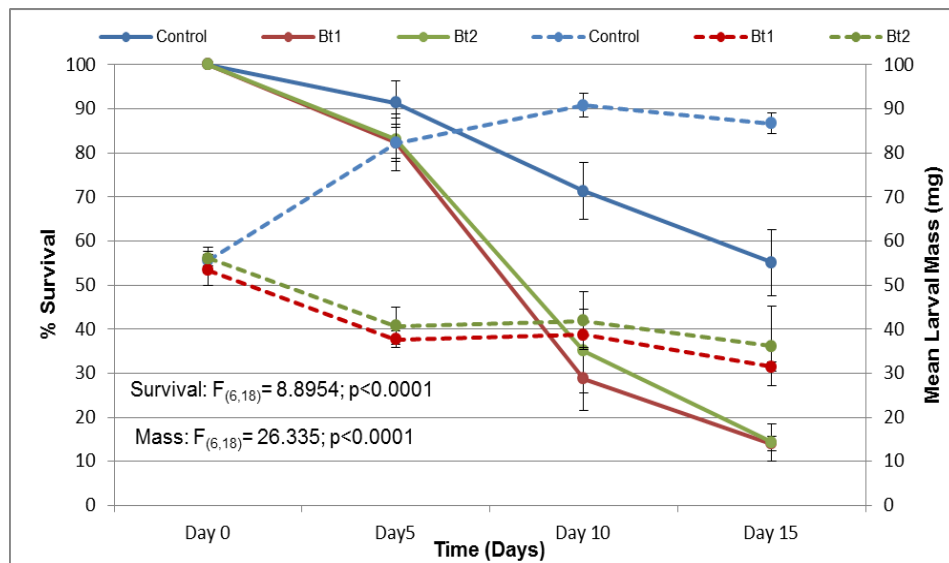


Figure 2-12: The mean percentage survival and mean mass of sixteen-day old *Chilo partellus* larvae over a 15-day period on Bt maize after transfer from non-Bt maize. (Vaalharts population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

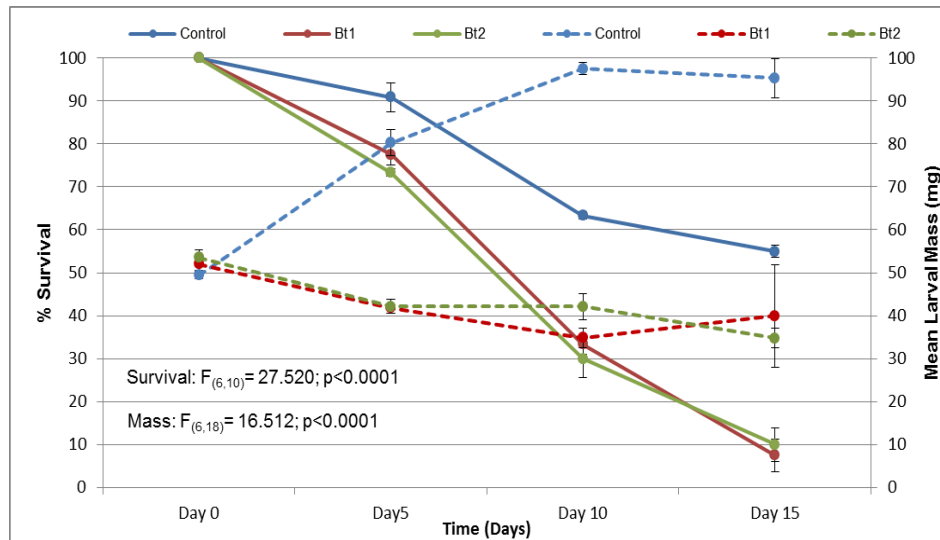


Figure 2-13: The mean percentage survival and mean mass of sixteen-day old *Chilo partellus* larvae over a 15-day period on Bt maize after transfer from non-Bt maize. (Vrededorf population) (Bars = SE). Solid lines indicate survival whereas dotted lines indicate mean mass. Non-Bt indicated in blue, Bt1 indicated in red and Bt2 indicated in green.

Data on larval survival and mean larval mass of neonate, seven- and 16- old larvae of the different *C. partellus* populations over time are provided in Table 2-1.

First instar larvae (1-day old larvae)

Significant differences were observed between both larval survival and mass on the non-Bt treatment and two Bt treatments, 21 days after commencing feeding on Bt leaf tissue (Table 2-1). However, there was no significant difference in larval survival between the two Bt treatments for any of the four populations. The Vaalharts population had the highest larval survival on non-Bt maize (58.67%), followed by the Potchefstroom population with 56.67%. The same was observed for larval mass of the Vaalharts and Potchefstroom populations with 90.19 and 88.13 mg.larva⁻¹ on non-Bt maize respectively.

Seven-day old larvae

There was a significant difference between the levels of survival on non-Bt and the two Bt treatments for each of the populations (Table 2-1). No significant differences were observed in larval survival between the two Bt treatments. Larvae of the Prieska population had the highest survival on non-Bt maize (73.61%), followed by the Potchefstroom population (66.30%) on day

14. There were significant differences between larval mass on the non-Bt and two Bt treatments for all four populations, but larval mass did not differ between the two Bt treatments.

Sixteen-day old larvae

Larval survival on non-Bt maize was significantly higher than on the two Bt treatments for all four populations while no significant differences were observed between survival on the Bt treatments (Table 2.1). However, larvae of all four populations survived on maize whorl tissue of both Bt treatments. The highest levels of survival on Bt maize were observed for the Prieska population with 32.7 and 28.4% on Bt1 and Bt2 respectively. There were significant differences between mean larval mass on non-Bt and the two Bt treatments for all four populations. No significant differences were observed between larval mass on the two Bt treatments for any of the four populations except for the Potchefstroom population.

Table 2-1: The mean percentage survival and mean larval mass of first instar-, seven- and 16-day old *Chilo partellus* larvae of the Potchefstroom, Prieska, Vaalharts and Vredefort populations after feeding on maize whorl tissue of non-Bt, Bt1 and Bt2 for a period 21, 14 and 15 days respectively.

	Inoculated as 1-day old larvae Data recorded after 21 days **				Inoculated as 7-day old larvae Data recorded after 14 days **				Inoculated as 16-day old larvae Data recorded after 15 days **			
	Survival (%)		Mass (mg)		Survival (%)		Mass (mg)		Survival (%)		Mass (mg)	
Potchefstroom												
Non-Bt→non-Bt	56.67 b		88.13 b		66.30 b		82.74 b		81.67 b		98.56 c	
Non-Bt→Bt 1	0.00 a		0.00 a		0.00 a		0.00 a		0.00 a		0.00 a	
Non-Bt→Bt 2	0.00 a		0.00 a		0.00 a		0.00 a		2.50 a		32.53 b	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
	95.07	0.00	1234.21	0.00	438.92	0.00	518.11	0.00	2329.75	0.00	57.70	0.00
Prieska												
Non-Bt→non-Bt	50.00 b		83.90 b		73.61 b		68.70 b		69.40 b		82.18 b	
Non-Bt→Bt 1	0.00 a		0.00 a		0.00 a		0.00 a		32.74 a		33.60 a	
Non-Bt→Bt 2	0.00 a		0.00 a		0.00 a		0.00 a		28.45 a		31.08 a	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
	89.29	0.00	418.72	0.00	10032.14	0.00	9588.11	0.00	13.58	0.01	380.13	0.00
Vaalharts												
Non-Bt→non-Bt	58.67 b		90.19 b		52.44 b		52.73 b		55.12 b		86.63 b	
Non-Bt→Bt 1	0.00 a		0.00 a		0.00 a		0.00 a		13.97 a		31.43 a	
Non-Bt→Bt 2	0.00 a		0.00 a		0.00 a		0.00 a		14.29 a		36.17 a	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
	138.78	0.00	479.84	0.00	263.96	0.00	43.16	0.00	21.45	0.00	31.95	0.00
Vredefort												
Non-Bt→non-Bt	42.67 b		79.78 b		56.17 b		53.07 b		55.00 b		95.25 b	
Non-Bt→Bt 1	0.00 a		0.00 a		0.00 a		0.00 a		7.50 a		40.03 a	
Non-Bt→Bt 2	0.00 a		0.00 a		0.00 a		0.00 a		10.00 a		34.80 a	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
	215.58	0.00	427.39	0.00	87.07	0.00	186.54	0.00	68.60	0.00	19.92	0.00

* Significant difference $p < 0.05$. (Means within columns followed by the same letter do not differ significantly).

** 1-day old larvae evaluated over a total of 21 days, 7-day old larvae over a total of 21 days and 16-day old larvae over a total of 31 days.

Table 2-2: Corrected mean percentage mortality for *Chilo partellus* larvae that fed for 16 days on non-Bt maize, before transfer to Bt maize for a period of 15 days.

Population	Corrected % Mortality			
	Treatment		t-value	p-value*
	Bt1	Bt2		
Potchefstroom	100 c**	97.06 a	1.73	0.16
Prieska	52.63 a	59.21 a	-0.53	0.62
Vaalharts	72.55 ab	72.55 a	0	1
Vredefort	86.36 bc	81.82 a	0.46	0.67
ANOVA	F= 20.67; p< 0.001	F= 3.57; p= 0.067		

*Means within a row were compared by means of t-tests, significant difference p<0.05.

** Means within columns followed by the same letter do not differ significantly.

There were no significant differences between the levels of larval mortality on the two Bt treatments for any of the four populations (Table 2-2). None of the 16-day old larvae of the Potchefstroom population survived on Bt1 after another 15 days of feeding. The larvae of the Prieska population had the lowest corrected percentage mortality on Bt1 after 15 days. There were no significant differences in larval mortality between the populations that had fed on Bt2

2.4.2 Evaluation of pupal development

Potchefstroom population

No larvae survived to the pupal stage on any of the two Bt treatments. A sex ratio of 1.77:1.0 (female: male) on the non-Bt treatment and a mean mass of 93.05 mg for the pupae (Table 2-3) were recorded. The pupation percentage was 68.8% on non-Bt maize, which was the second highest of the four populations.

Prieska population

The sex ratio was 0.41:1.0 on non-Bt, 0.09:1.0 on Bt1 and no females were recorded on Bt2 (Table 2-3). There were no significant differences between the sex ratios of pupae that developed on non-Bt and the two Bt maize treatments, or between the sex ratios of pupae that developed on the two Bt treatments. There was a significant difference between mean pupal mass on non-Bt and the two Bt treatments and no significant difference between the two Bt treatments. The percentage pupation on the different treatments ranged between 10.91 and 75.45% respectively for Bt1 and non-Bt.

Vaalharts population

The sex ratio was 0.36:1.0 on non-Bt maize and no female pupae were recovered from either of the Bt treatments (Table 2-3). There was a significant difference between the mean pupal mass on the non-Bt and Bt treatments but no significant difference between that on the Bt1 and Bt2 treatments. The pupation percentage was 64.55% on non-Bt, 11% on Bt1 and 4.26 % on Bt2.

Vredefort population

There was a significant difference in the sex ratio of pupae that developed on non-Bt maize and the two Bt treatments but no difference between the sex ratio on the two Bt treatments, since these two only produced males (Table 2-3). There was a significant difference between the mean pupal mass of larvae that developed on non-Bt and Bt1 maize but not between non-Bt and Bt2. The pupation rates were 56.67% on non-Bt, 9.17% on Bt1 and 5.83% on Bt2 maize.

Table 2-3: The sex ratio, mean pupa mass and pupation (%) of the 16-day old larvae of *Chilo partellus* that developed into pupae.

Population	Treatment	Sex ratios		Mean pupal mass		Pupation % (number of pupae)		
Potchefstroom	Non-Bt	1:1.77		93.05		68.8 (86)		
	Bt1	-		-		0 (0)		
	Bt2	-		-		0 (0)		
		Chi	p-value	t-value	p-value	Chi	p-value*	
		Non-Bt vs Bt1	-	-	-	-	128.07	0.00
		Non-Bt vs Bt2	-	-	-	-	128.07	0.00
	Bt1 vs Bt2	-	-	-	-			
Prieska	Non-Bt	1:0.41		76.40		75.45 (83)		
	Bt1	1:0.09		35.56		10.91 (12)		
	Bt2	1:0		32.27		11.82 (13)		
		Chi	p-value	t-value	p-value	Chi	p-value	
		Non-Bt vs Bt1	1.35	0.25	4.34	0.00	90.78	0.00
		Non-Bt vs Bt2	3.59	0.06	4.88	0.00	87.99	0.00
	Bt1 vs Bt2	0.00	0.97	0.95	0.35	0.00	1.00	
Vaalharts	Non-Bt	1:0.36		81.38		64.65 (64)		
	Bt1	1:0		44.5		11 (11)		
	Bt2	1:0		33.7		4.26 (4)		
		Chi	p-value	t-value	p-value	Chi	p-value	
		Non-Bt vs Bt1	2.41	0.12	3.76	0.00	58.70	0.00
		Non-Bt vs Bt2	0.35	0.55	2.96	0.00	74.44	0.00
	Bt1 vs Bt2	-	-	1.72	0.11	2.22	0.14	
Vredefort	Non-Bt	1:0.84		87.41		56.67 (68)		
	Bt1	1:0		38.53		9.17 (11)		
	Bt2	1:0		47.24		5.83 (7)		
		Chi	p-value	t-value	p-value	Chi	p-value	
		Non-Bt vs Bt1	6.45	0.01	2.19	0.03	59.17	0.00
		Non-Bt vs Bt2	3.72	0.05	1.43	0.16	69.82	0.00
	Bt1 vs Bt2	-	-	2.39	0.03	0.54	0.46	

* Means within columns were compared by means of t-tests and Chi-square tests, significant difference p<0.05

2.5 Discussion

No first instar larvae survived on any of the Bt treatments. Survival of 7.71% and 2.3% on Bt1 and Bt2 respectively were recorded seven days after 7-day old larvae were transferred to Bt maize, but no live larvae were recovered at day 14. This may indicate that the larvae have some tolerance to the different Bt maize events used in this study. Much higher survival was observed when 16-day old larvae were transferred to Bt1 and Bt2 maize leaf tissue where they fed for another 15 days with survival of 32.74% and 28.45 % recorded on Bt 1 and Bt 2 respectively. This indicates that larvae from the Prieska population may have developed a low level of tolerance to Bt maize.

Some larvae of the Prieska, Vaalharts and Vredefort populations were able to develop into pupa on the two Bt maize events. This indicates that a small number of *C. partellus* larvae would be able to complete their life cycle on Bt maize if they were able to migrate from a non-Bt maize plant to a Bt maize plant when the larvae are 16 days and older. This indicates that the larvae have a level of tolerance to the Cry1Ab toxin when they are older. Where a resistant female survives such as in the case of Bt1 for the Prieska population (Table 2-3), there may be a chance that a proportion of the F1-generation larvae may be resistant to the Bt maize. The other two populations produced only male pupa on the two Bt treatments. However, if there were a higher percentage survival more females may have been produced. Only one female moth of the Prieska population was able to mate and produce eggs but no further studies were done on the offspring. Under field conditions, such events may lead to an increase in heterozygote fitness and selection pressure that will increase the risk of resistance development.

The low levels of survival observed in this study are similar to that reported by Mugo *et al.* (2011) for *C. partellus* on Bt-incorporated artificial diets in Kenya. Significantly lower survival of *B. fusca* and *C. partellus* larvae (Mugo *et al.*, 2005) on Bt MON810 hybrids compared to the non-Bt hybrid and the public Bt Event 223 was also reported from Kenya. The mass of the larvae on the Bt MON810 hybrids reported by Mugo *et al.* (2011) are similar to the low mass observed in this study. This also correlates with the findings of Van Rensburg (1999) in South Africa with Bt maize events MON800, MON 801 and MON802 after 9 days of larval feeding. However, even though Mugo *et al.* (2011) observed good levels of control of *C. partellus* on various Bt maize events, significant damage to plants were also recorded. This indicates higher levels of survival of *C. partellus* on the Bt events used in their study.

2.6 Conclusions

When first instar larvae were inoculated directly onto tissue of the two Bt maize events, larvae died within the first seven days, indicating high levels of susceptibility of this species collected from four different localities. Almost the same tendency was observed when larvae were seven days old and all of them died within 14 days on Bt maize. A small percentage of the 16-day old larvae survived on Bt maize whorl tissue. This chapter therefore addressed the research question on whether or not larval age affected survival on Bt maize.

It can be concluded that migrating 16-day old larvae have a higher possibility of survival on Bt maize. Thus if older larvae migrate from a non-Bt maize plant to a Bt maize plant, the larvae may survive and have the ability to cause significant damage to plants. Some of the sixteen-day old larvae completed their life cycle and could therefore, as moths, introduce resistance genes to the next generation.

This study indicates that seed mixture plantings will not be ideal and it may actually contribute towards resistance development. Carroll *et al.* (2012) stated that if susceptible larvae migrate from non-Bt plants to Bt plants, the efficiency of the refuge is reduced. The optimum future IRM strategy will most likely be to continue using the high/dose refuge strategy as it is currently used in South Africa. It is strongly recommended that *C. partellus* populations be monitored for resistance to detect early shifts in the levels of susceptibility.

2.7 References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Archer, T.L., Patrick, C., Schuster, G., Cronhom, G., Bynum Jr., E.D. & Morrison, W.P. 2001. Ear and shank damage by corn borers and corn earworms to four events of *Bacillus thuringiensis* transgenic maize. *Crop Protection*, 20: 139-144.

Carroll, M.W., Head, G. & Caprio, M. 2012. When and where a seed mix refuge makes sense for managing insect resistance to Bt plants. *Crop Protection*, 38: 74-79.

Gouse, M., Pray, C.E., Kirsten, J. & Schimmelpfennig, D. 2005. A GM subsistence crop in Africa: the case of Bt white maize in South Africa. *International Journal of Biotechnology*, 7: 84-94.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa, M., Songa, J. & Gichuki, S. 2005. Developing Bt maize for resource-poor farmers- recent advances in the IRMA project. *African Journal of Biotechnology*, 4: 1490-1504.

Mugo, S., Murenga, M.G., Karaya, H., Tende, R., Taracha, C., Gichuki, S., Ininda, J., M'bijjewe, K. & Chavangi, A. 2011. Control of *Busseola fusca* and *Chilo partellus* stem borers by *Bacillus thuringiensis* (Bt)- δ -endotoxins from Cry1Ab gene Event MON810 in greenhouse containment trials. *African Journal of Biotechnology*, 10: 4719-4724.

Van den Berg, J., Hilbeck, A. & Böhn, T. 2013. Pest resistance to Cry1Ab Bt maize: field resistance, contributing factors and lessons from South Africa. *Crop Protection*, 54: 154-160.

Van Rensburg, J.B.J. 1999. Evaluation of Bt-transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant and Soil*, 16: 38-43.

Van Rensburg, J.B.J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil*, 24: 147-151.

Yang, F., Kerns, D.L., Head, G.P., Leonard, B.R., Levy, R., Niu, Y. & Huang, F. 2014. A challenge for the seed mixture refuge strategy in Bt maize: impact of cross pollination on an ear-feeding pest, corn earworm. *PLoS ONE*, 9: e112962 doi:10.1371/journal.pone.0112962.

CHAPTER 3

THE EFFECT OF SHORT-TERM EXPOSURE TO BT MAIZE ON *CHILO PARTELLUS* LARVAL SURVIVAL AND GROWTH

3.1 Abstract

Over the years many lepidopteran pests have developed resistance to transgenic Bt crops. In South Africa the African maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae), developed resistance to the Bt event with the Cry1Ab gene, only eight years after the first release of Bt maize. However, the spotted stem borer *Chilo partellus* (Lepidoptera: Crambidae), which is also of economic importance on maize, have not developed resistance to Bt maize in South Africa. In this study larvae of a *C. partellus* population were exposed to Bt maize and surviving moths used to generate a F1 and F2 generation, to determine if selection for tolerance in a population could be done under laboratory conditions. Since it is known that *C. partellus* is highly susceptible to Cry1Ab toxin and that larval mortality on Bt maize is very high, neonate larvae of the first generation was only allowed to feed on Bt maize for one day after which they were transferred back to non-Bt maize to complete their life cycle. Surviving adults of this F2 generation were then mated and their neonate larvae fed on Bt maize for two days before transferring them back to non-Bt maize. Larval survival and larval mass were determined as well as the sex ratio, pupal mass, percentage pupation and the pupal development time for each of the F0 and F1 generations. Larvae of the F1 generation that fed on Bt maize had a higher mean larval mass than those of the F0 generation and F1 generation larvae which have fed on non-Bt maize tissue. Female pupa of the F1 generation larvae that were exposed to Bt maize had a higher mass than that of the F0 and F1 generation's larvae that had previously fed on non-Bt. Larva to pupa development rates were faster for the F1 generation exposed to Bt maize tissue. The observed increase in larval survival and mass of the F1 population, although very small, indicated that short-term feeding on Bt maize prior to larval movement to adjacent plants that may be non-Bt, may contribute to larval survival in mixed seed systems.

Keywords: Bt maize, *Chilo partellus*, resistance

3.2 Introduction

Insect pests that develop resistance to Bt toxins are a major concern worldwide. Over the past decade several pest species evolved resistance to these Bt crops (Tabashnik *et al.*, 2013). Reports of field evolved resistance to Bt have been documented for lepidopteran pests such as the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) (Tabashnik *et al.*, 1990), the African maize stem borer *Busseola fusca* (Lepidoptera: Noctuidae) (Van Rensburg, 2007), the Fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Farias *et al.*, 2014), the pink bollworm *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) (Dhurua & Gujar, 2009) and the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae) (Janmaat *et al.*, 2004).

In South Africa insect resistance evolution to Bt maize is a major concern, since this is the staple food in the country. The first insect resistant maize was planted in 1998 and during 2016, 2.16 million hectares of transgenic maize were planted of which 19.5% was insect resistant and 61.7% were of the pyramided traits consisting of insect resistance and herbicide tolerance (James, 2016). *Busseola fusca* developed resistance to the Bt event with the Cry1Ab gene, only eight years after the first release of Bt maize in South Africa (Van Rensburg, 2007). The other target pest of Bt maize, the spotted stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) has however not been reported to be resistant and no reports from farmers have been made in this regard.

The fact that *C. partellus* has not evolved resistance to Bt maize over the past 19 years, is ascribed to its inherent high susceptibility to the Cry1Ab toxin (Van Rensburg, 1999). However, resistance evolution remains a threat and it is most likely only a matter of time until resistant populations are reported from the field. Selection for resistance under laboratory conditions can assist to identify biological markers that indicate the presence of resistance genes in stem borers (Crespo *et al.*, 2010). This can assist researchers in monitoring of resistance levels in pest populations over time, and provide early warning, in order to develop mitigation strategies.

In this study the effects of exposure of *C. partellus* larvae to Bt toxin over generations were determined and the effects on larval development and pupa development were compared between generations.

3.3 Materials and methods

Life history parameters of a *C. partellus* population were compared after selection for resistance over one generation and mating of offspring to produce a follow up generation. Selection for Bt-tolerant individuals were made by feeding them with Bt maize leaf tissue for a short period and rearing of the survivors on non-Bt maize until they completed their life cycle. The Bt maize events used were Bt maize expressing Cry1Ab protein, or stacked-gene Bt maize expressing Cry1A.105 and Cry2Ab2 proteins. The latter two types of Bt maize are referred to as Bt1 and Bt2 respectively.

The *C. partellus* larvae used in this study originated from a source population that is maintained at the ARC-Grain Crops Institute in Potchefstroom where it has been reared on artificial diet for 18 generations prior to this study.

3.3.1 Parental generation (F0 generation)

Neonate larvae (1500) from eggs of moths from the source population constituted as the F0 generation for this experiment. Larvae were inoculated onto maize whorl tissue in 100 ml containers of the two Bt treatments. Each container represented a replicate and the number of replicates were 58 and 72 for Bt1 and Bt2, respectively. Ten larvae were inoculated into each container.

Larvae were allowed to feed for only 24 hrs after which they were transferred onto non-Bt maize whorl tissue in similar containers. Larvae were reared for the rest of their life cycle on non-Bt maize.

Larval survival and mass were determined on day 7, 14 and 21 and fresh non-Bt maize whorl tissue was provided at each of these intervals until the pupal stage was reached. Pupal mass and sex were determined and the pupae were placed into steel meshed cages (37 cm in length and 23 cm in diameter) with wax paper as oviposition substrate for emerging moths and a sugar solution for moths to drink from. Eggs were collected from the cages and sterilized by suspending it in a 2% sodium hypochlorite solution. Eggs were then incubated at $27\pm 2^{\circ}\text{C}$ at 40 to 70% relative humidity. The neonate larvae that hatched from these eggs were used as F1 generation larvae.

3.3.2 F1 generation

Survival and performance of F1 generation larvae on Bt1 maize leaf tissue

Neonate larvae that hatched from eggs laid by the F0 generation moths were used in this experiment. These larvae were divided into three groups (treatments) and designated as follows: larvae put onto non-Bt maize for its whole life cycle (F1-Bt1-Control), larvae that fed for one day on Bt1 maize before transfer to non-Bt maize (F1-Bt1-1day) and larvae that fed on Bt maize for two days before transfer to non-Bt maize to complete their cycle (F1-Bt1-2days). A flow diagram indicating the different treatments and sequence of events is presented in Figure 3-1. Each container represented a replicate and replicate numbers were as follows: 35 for the F1-Bt1-Control, 94 for F1-Bt1-1day and 98 for F1-Bt1- 2days treatments.

For each treatment ten neonate larvae were inoculated onto the maize whorl tissue in small 100 ml containers and left to feed until the pupal stage was reached. Larval survival and mass was determined on day 7, 14 and 21 and fresh non-Bt maize whorl tissue was provided at each of these intervals. Pupal mass and sex was determined and the pupae were then placed into steel mesh cages with wax paper as described above. Emerging moths were allowed to mate and lay eggs. Eggs were sterilized and maintained in a temperature controlled room as described above. Larvae that hatched from these eggs are referred to as the F2 generation. These F2 generation larvae were however not further subjected to selection pressure for resistance, due to time constraints and the approximate 2 months needed to complete one generation.

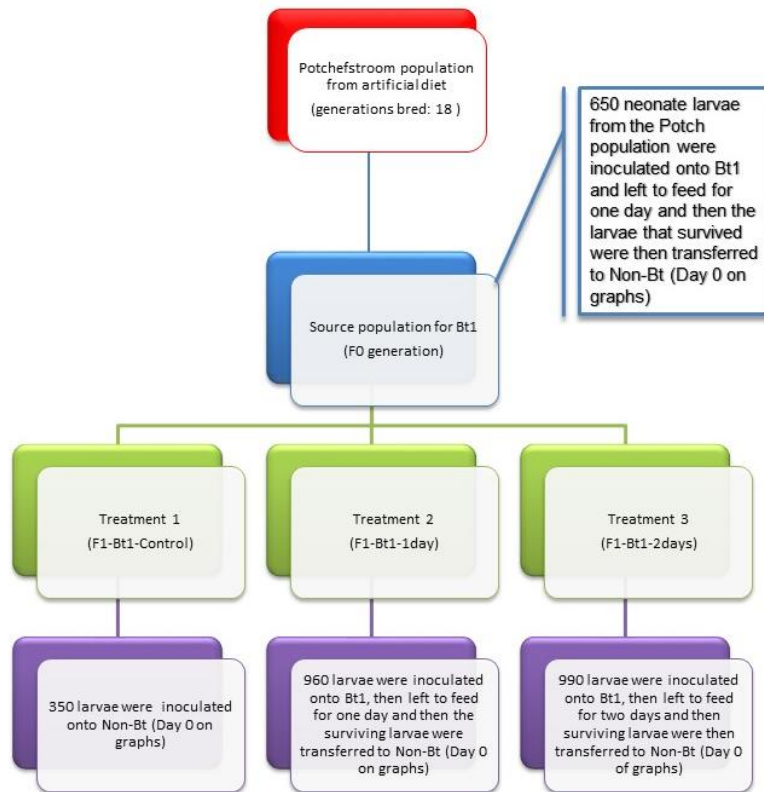


Figure 3-1: A schematic representation of how the experiment was conducted with the *Chilo partellus* population on Bt1 maize. Treatment 1 indicates the F1 generation larvae which fed on non-Bt maize, treatment 2 indicates the F1 generation larvae which fed on Bt1 maize for one day and treatment 3 indicates the F1 generation larvae which fed on Bt1 maize for 2 days. (Source population = F0 generation).

Survival and performance of F1 generation larvae on Bt2 maize leaf tissue

Neonate larvae that hatched from eggs laid by the F0 generation moths were used in this experiment. These larvae were divided into three groups (treatments) and designated as follows: larvae put onto non-Bt maize for its whole life cycle (F1-Bt2-Control), larvae that fed one day on Bt2 maize before transfer to non-Bt maize (F1-Bt2-1day) and larvae that fed on Bt maize for two days before transfer to non-Bt maize to complete their cycle (F1-Bt2-2days). A flow diagram indicating the different treatments and sequence of events is presented in Figure 3-2. Each container represented a replicate and the number of replicates for each treatment were as follows: 40 for the F1-Bt2-Control, 44 for the F1-Bt2-1day treatment and 48 for the F1-Bt2-2days treatment.

For each treatment ten neonate larvae were inoculated onto the maize whorl tissue in small 100 ml containers and left to feed till the pupal stage was reached. Larval survival and mass was

determined on day 7, 14 and 21, when fresh non-Bt maize whorl tissue was provided at each of these intervals. Pupal mass and sex was determined after which the pupae were placed into steel mesh cages with wax paper as described above. Eggs were sterilized and maintained in a temperature controlled room as described above. Larvae that hatched from these eggs are referred to as the F2 generation. These F2 generation larvae were however not further subjected to selection pressure for resistance, due to the constraints described above.

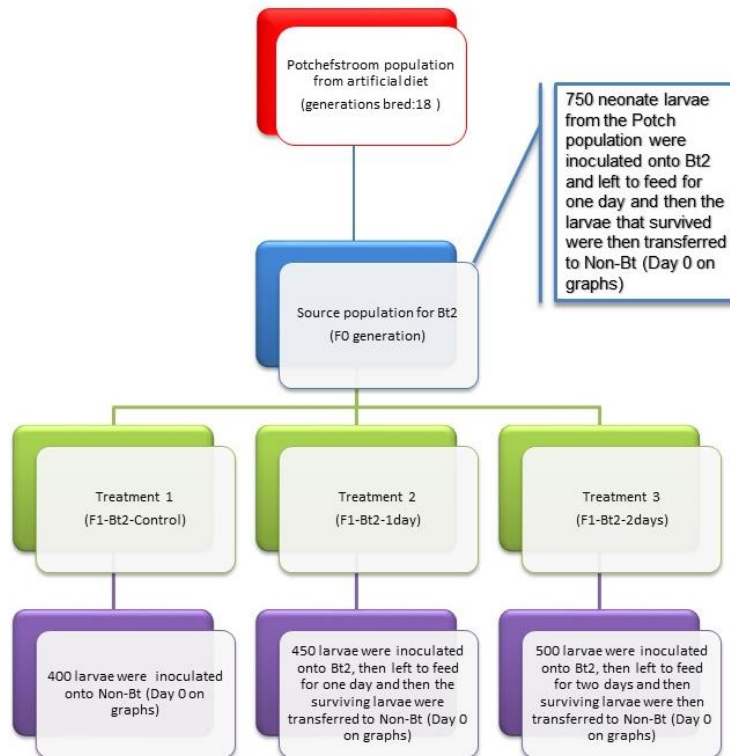


Figure 3-2: A schematic representation of how the experiment was conducted for *Chilo partellus* population on Bt2 maize. Treatment 1 indicates the F1 generation larvae which fed on non-Bt maize, treatment 2 indicates the F1 generation larvae which fed on Bt2 maize for one day and treatment 3 indicates the F1 generation larvae which fed on Bt2 maize for 2 days. (Source population = F0 generation).

Pupae F0 and F1 generations for Bt1 and Bt2

For each of the above two F1 the number of pupae that developed on each treatment was determined at 28, 35 and 42 days after transfer of larvae back to non-Bt maize tissue to complete their life cycles.

The cumulative number of pupae were determined and expressed as a percentage of the number of individuals in that treatment.

Data analysis

Data were analysed using Genstat edition 18 and SPSS version 24. Data on larval survival and mass was analysed by means of Student t-tests to compare between generations and treatments. Pupal mass and percentage pupation was analysed by means of Student t-test and sex ratio by means of a Chi-square test. DT_{50} values, indicating the time (in number of days) until 50% of larvae of each treatment pupated, were determined by means of Probit analyses.

3.4 Results

Larval mass and survival

F0 generation larvae exposed to Bt maize for only one day had a mean survival of 89.2 and 96.0% on Bt1 and Bt2 maize tissue respectively, before being transferred to non-Bt maize (Table 3-1). The F1 larvae (offspring of the F0 generation) which fed on Bt1 maize tissue for one day had a 97.08% survival while those that fed for 2 days on Bt1 maize tissue had a survival of 98.13% before being transferred to non-Bt maize (Table 3-1). The F1 larvae that were given the same treatment as F0 population on Bt2 had a 97.78% survival whereas the other that fed on Bt2 for 2 days had a survival of 95% before being transferred to non-Bt maize tissue (Table 3-1).

Table 3-1: Selection process to determine resistance and survival for *Chilo partellus* over generations under laboratory conditions.

Generation	Generation name	Type of maize provided as food	Initial number of neonates	Number of days on Bt	Larval survival (%) after feeding on Bt maize for 1 or 2 days
F0	Source population	Bt1	650	1	89.23
	Source population	Bt2	750	1	96.00
F1	F1-Bt1-Control	Non-Bt	350	0	100
	F1-Bt1-1day	Bt1	960	1	97.08
	F1-Bt1-2days	Bt1	990	2	98.13
	F1-Bt2-Control	Non-Bt	400	0	100
	F1-Bt2-1day	Bt2	450	1	97.78
	F1-Bt2-2days	Bt2	500	2	95.00

Survival and performance on Bt1

Survival of F0 larvae (source population) that previously fed on Bt1 maize for one day and its offspring (F1 generation) exposed to three different treatments, decreased over the 21-day experiment period on the different treatments. Larval survival on day 21 ranged between 20 and 38% for the different treatments (Figure 3-4).

Larval mass increased, although larval survival decreased for the source population and its offspring on Bt1. The source population (initially fed on Bt1 maize tissue for 1 day) had a survival of 32.24%, compared to the 20.92% (1-day) and 21.68% (2-days) of its offspring which was exposed to Bt1 maize (Table 3-2). Offspring that fed only on non-Bt maize tissue had a higher survival rate than the rest of the offspring which had fed on Bt maize. There were no significant differences between the survival of the offspring that fed on Bt1 maize tissue. Offspring that fed on Bt1 maize tissue for one day not only had a lower survival than the source population, but also a lower larval mass. Larval mass of the offspring (F1 generation) that fed for 2 days on Bt1 maize tissue did not differ significantly from that of the source population. The larvae that fed on Bt1 maize tissue for 2 days had a higher mass than that of the larvae which had fed only for 1 day.

Survival and performance on Bt2

After larvae were transferred onto non-Bt maize tissue, their survival decreased over the following 21 days. Larval survival ranged between 5 and 30% on the different treatments, including the control (Figure 3-5) and decreased over the 21-day period.

Larvae which fed on non-Bt maize tissue had the highest survival (30.25%) while offspring of the source population which fed on Bt2 maize tissue for 2 days had the lowest survival (4.79%) on day 21 (Table 3-3). There were no significant differences between survival of the F1 offspring and that of the source population on the same treatment. The larvae that fed for 1 day on Bt2 maize tissue before transfer to non-Bt maize, had the lowest larval mass after 21 days (Table 3-3), whereas the offspring which fed only on non-Bt maize tissue had the highest larval mass. Offspring of the F0 generation that fed on Bt2 maize tissue for 2 days before transfer to non-Bt maize had a higher mass after 21 days than the offspring that fed for one day before transfer but it did not differ significantly from that of the source population.

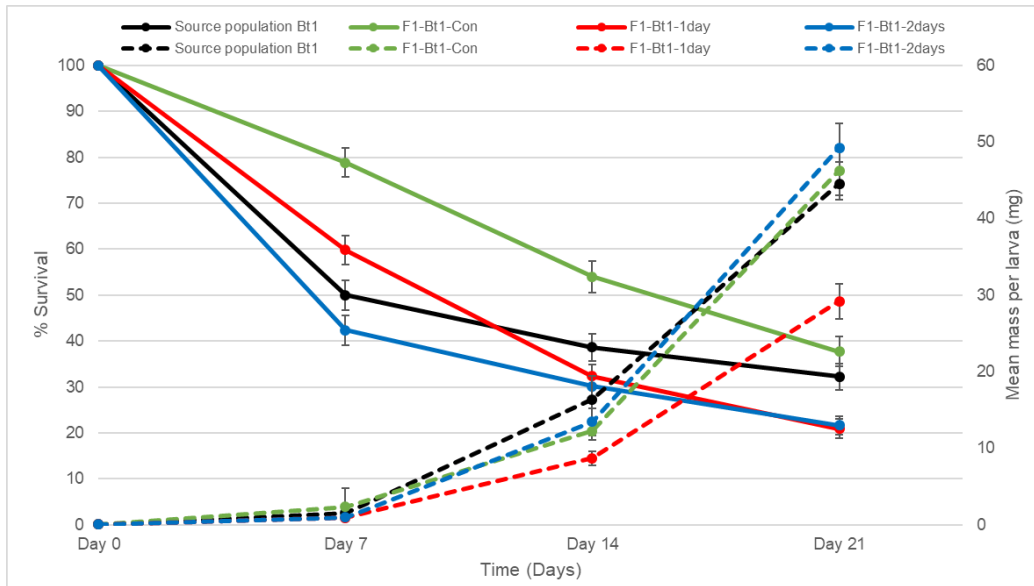


Figure 3-4: The mean percentage survival and mean mass over 21 days for *Chilo partellus* larvae that fed on Bt1 and non-Bt maize (Bars= Standard Error (SE)). Solid lines indicate larval survival whereas dotted lines indicate mean larval mass. Black lines indicate the source population (F0) feeding on Bt1 for 1 day. Green lines indicate F1 generation larvae that fed only on non-Bt maize. Red lines indicate F1 generation larvae that fed on Bt1 for 1 day. Blue lines indicate F1 generation larvae that fed on Bt1 for 2 days.

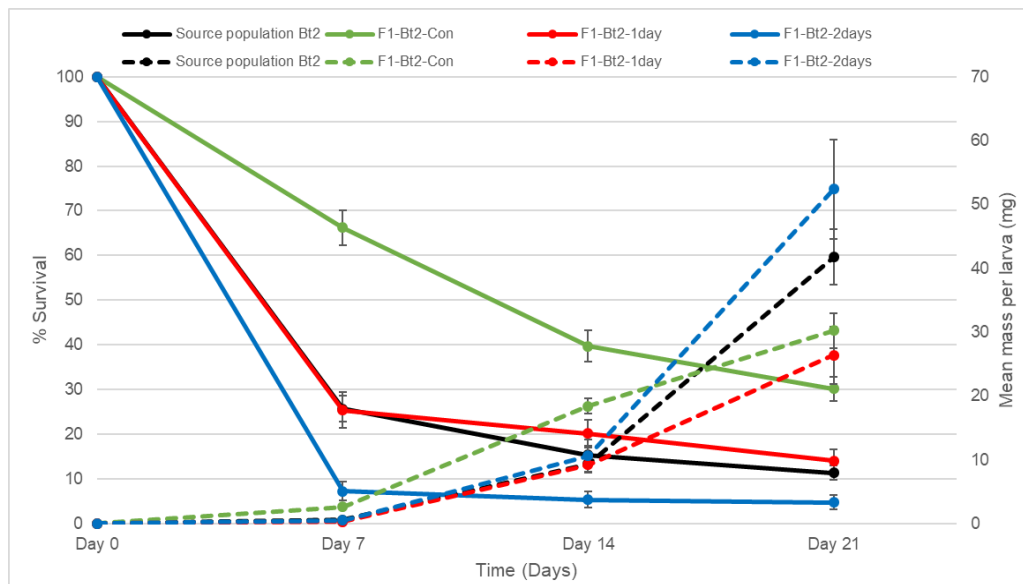


Figure 3-5: The mean percentage survival and mean mass over 21 days for *Chilo partellus* larvae that fed on Bt2 and non-Bt maize (Bars= Standard Error (SE)). Solid lines indicate larval survival whereas dotted lines indicate mean larval mass. Black lines indicate the source populations (F0) feeding on Bt2 for 1 day. Green lines indicate F1 generation larvae feeding only on non-Bt maize. Red lines indicate F1 generation larvae that fed on Bt2 for 1 day. Blue lines indicate F1 generation larvae that fed on Bt2 for 2 days.

Table 3-2: The mean percentage survival and larval mass of *Chilo partellus* larvae feeding on Bt1 and non-Bt maize for 21 days under laboratory conditions. SE = Standard Error of the means.

	% Survival on day 21		Mean larval mass on day 21	
F0 population on Bt1	32.24 (+SE 2.89)		44.52 (+SE 2.05)	
F1-Bt1-Con	37.71 (+SE 3.20)		46.26 (+SE 3.23)	
F1-Bt1-1day	20.92 (+SE 1.60)		29.17 (+SE 1.79)	
F1-Bt1-2days	21.68 (+SE 1.62)		49.23 (+SE 2.13)	
	t-value	p-value*	t-value	p-value
F0 population vs F1-Bt1-Con	-1.268	0.208	-0.456	0.650
F0 population vs F1-Bt1-1day	3.425	0.001	5.627	0.000
F0 population vs F1-Bt1-2days	3.187	0.002	-1.600	0.112
F1-Bt1-Con vs F1-Bt1-1day	4.687	0.000	4.622	0.000
F1-Bt1-Con vs F1-Bt1-2days	4.465	0.000	-0.771	0.443
F1-Bt1-1day vs F1-Bt1-2days	-0.334	0.739	-7.214	0.000

Table 3-3: The mean percentage larval survival and larval mass of *Chilo partellus* larvae feeding on Bt2 and non-Bt maize for 21 days under laboratory conditions. SE = Standard Error of the means.

	% Survival on day 21		Mean larval mass on day 21	
F0 population Bt2	11.39 (+SE 1.43)		41.78 (+SE 3.00)	
F1-Bt2-Con	30.25 (+SE 2.69)		67.20 (+SE 2.75)	
F1-Bt2-1day	14.09 (+SE 2.28)		26.38 (+SE 4.06)	
F1-Bt2-2days	4.79 (+SE 1.43)		52.39 (+SE 6.23)	
	t-value	p-value	t-value	p-value
F0 population vs F1-Bt2-Con	-6.189	0.000	-6.241	0.000
F0 population vs F1-Bt2-1day	-1.003	0.319	3.051	0.003
F0 population vs F1-Bt2-2days	3.264	0.001	-1.533	0.145
F1-Bt2-Con vs F1-Bt2-1day	4.579	0.000	8.324	0.000
F1-Bt2-Con vs F1-Bt2-2days	8.357	0.000	2.173	0.045
F1-Bt2-1day vs F1-Bt2-2days	3.453	0.001	-3.497	0.002

Table 3-4: The sex ratio, mean pupal mass and pupation (%) of *Chilo partellus* larvae that fed on Bt1 and non-Bt maize and which developed into pupae under laboratory conditions. SE = Standard Error of the means.

	Sex ratio (Male: Female)		Pupal mass				% Pupation (number of pupae)	
			Female		Male			
F0 population Bt1	1:0.81		95.13 (+SE 2.47)		47.12 (+SE 1.77)		20.00 (116)	
F1-Bt1-Con	1:0.57		102.71 (+SE 5.42)		54.38 (+SE 1.99)		21.14 (74)	
F1-Bt1-1day	1:0.85		130.15 (+SE 4.63)		60.75 (+SE 2.12)		9.77 (87)	
F1-Bt1-2days	1:0.90		117.86 (+SE 3.68)		51.59 (+SE 1.72)		11.22 (110)	
	Chi-square value	p-value*	t-value	p-value	t-value	p-value	t-value	p-value
F0 population vs F1-Bt1-Con	1.290	0.255	-1.273	0.211	-2.723	0.008	-0.372	0.711
F0 population vs F1-Bt1-1day	0.030	0.871	-6.472	0.000	-4.994	0.000	4.592	0.000
F0 population vs F1-Bt1-2days	0.140	0.712	-5.131	0.000	-1.767	0.080	3.765	0.000
F1-Bt1-Con vs F1-Bt1-1day	1.480	0.223	-3.792	0.000	-1.983	0.050	4.852	0.000
F1-Bt1-Con vs F1-Bt1-2days	2.100	0.147	-2.313	0.025	1.036	0.303	4.043	0.000
F1-Bt1-1day vs F1-Bt1-2days	0.030	0.856	2.009	0.048	3.068	0.003	-1.362	0.175

Table 3-5: The sex ratio, mean pupal mass and pupation (%) of *Chilo partellus* larvae that fed onto Bt2 and non-Bt maize and which developed into pupae under laboratory conditions. SE = Standard Error of the means.

	Sex ratio (Male: Female)		Pupal mass				% Pupation (number of pupae)	
			Female		Male			
F0 population Bt2	1:0.73		88.85 (+SE 4.17)		51.86 (+SE 2.49)		7.22 (52)	
F1-Bt2-Con	1:0.95		92.98 (+SE 3.24)		42.86 (+SE 2.00)		20.00 (80)	
F1-Bt2-1day	1:0.71		118.48 (+SE 9.27)		53.04 (+SE 3.02)		6.59 (29)	
F1-Bt2-2days	1:0.46		138.13 (+SE 10.31)		52.89 (+SE 3.51)		4 (19)	
	Chi-square value	p-value*	t-value	p-value	t-value	p-value	t-value	p-value
F0 population vs F1-Bt2-Con	0.440	0.510	-0.781	0.439	2.816	0.007	-6.450	0.000
F0 population vs F1-Bt2-1day	0.010	0.935	-2.914	0.010	-0.301	0.765	0.393	0.695
F0 population vs F1-Bt2-2days	0.670	0.413	-4.420	0.003	-0.240	0.812	2.030	0.045
F1-Bt2-Con vs F1-Bt2-1day	0.460	0.496	-2.596	0.021	-2.808	0.009	6.471	0.000
F1-Bt2-Con vs F1-Bt2-2days	1.830	0.177	-4.178	0.006	-2.484	0.022	7.732	0.000
F1-Bt2-1day vs F1-Bt2-2days	0.470	0.493	-1.418	0.180	0.032	0.975	1.531	0.129

Pupal development of larvae that fed on Bt1

No significant differences were observed between the sex ratios of the F0 and F1 generations (Table 3-4). The mean mass of female pupae of the source population was significantly lower than that of its offspring of which the larvae fed on Bt1 maize (Table 3-4). There was no significant difference between female pupal mass of the source population and that of its offspring which was reared on non-Bt maize. Offspring that fed on Bt1 maize tissue for 1 day and then completed their cycle on non-Bt maize had the highest mean female pupal mass, followed by the offspring which fed on Bt1 maize tissue for 2 days before transfer. The source population and its offspring that fed only on non-Bt maize tissue until pupation had a higher mean percentage pupation rate than that of the offspring which initially fed on Bt1 maize tissue (Table 3-4).

Pupal development of larvae that fed on Bt2 maize

There were no significant differences between the sex ratios of the F0 and F1 generation pupae (Table 3-5). Female pupae of the source population (F0) had a lower mass than the offspring (F1) which had fed on Bt2 maize tissue and then completed their cycle on non-Bt maize (Table 3-5). No differences were observed between female pupal mass of the source population and its offspring which fed on non-Bt maize tissue. Offspring that fed on Bt2 maize tissue prior to transfer had a higher female pupal mass than the offspring which fed on non-Bt tissue. No significant difference was observed between the female pupal mass of the offspring which fed on Bt2 tissue, even though the offspring that fed on Bt2 for 2 days had a higher female pupa mass. Offspring that fed on only on non-Bt maize tissue until they pupated had the highest mean percentage pupation (Table 3-5). Larvae which fed on Bt2 maize tissue for 2 days had the lowest mean percentage pupation, but it did not differ significantly from that of the offspring which fed only for one day on Bt2 maize tissue.

Pupa formation

Larvae started to pupate after 21 days. On day 28, between 5 and 15% pupa had formed for the two generations that were reared on Bt1 maize ((Fig. 3-6 (a)). On day 35, pupa formation ranged between 28 and 56% for the two generations on Bt1 (Fig. 3-6 (a)). For the two generations on Bt2 maize, pupa formation ranged between 8 and 57% on day 28 (Fig. 3-6 (b)) and 28 and 78% on day 35.

The number of days until 50% pupa formation is indicated in Table 3-6. The duration of the period until 50% pupa formation when larvae fed on Bt1 maize tissue for one day ranged between 35.77 and 40.14 days. Larvae of the F0 population which fed on Bt1 maize tissue for the first time and only one day (before being transferred to non-Bt maize tissue) developed to the pupal stage slower than its offspring which fed on Bt1 maize tissue for 2 days before they were transferred (3.09 days)

(Table 3-6). Offspring of the source population's individuals that survived on Bt1 after feeding on it for 1 day took on average 39.72 days to reach 50% pupa formation, which is similar to the source population's 40.17 days. The offspring of the source population which fed on non-Bt took 35.77 days till 50% pupa formation. However, offspring of the source population which was exposed to Bt1 for 2 days took 37.0 days, which is on average 2.6 days faster than the larvae which fed on Bt1 for 1 day. Duration until 50% pupa formation of larvae that fed on Bt2 maize tissue ranged between 29.5 and 40.4 days. Larvae of the source population which were given the same treatment as the source population on Bt2 maize tissue took on average seven days longer to develop to pupae than larvae that first fed for 2 days on Bt2 maize tissue. Offspring of the source population which had fed for 1 day on Bt2 maize took 3.3 days less than that of the source population for 50% of the larvae to develop to pupae.

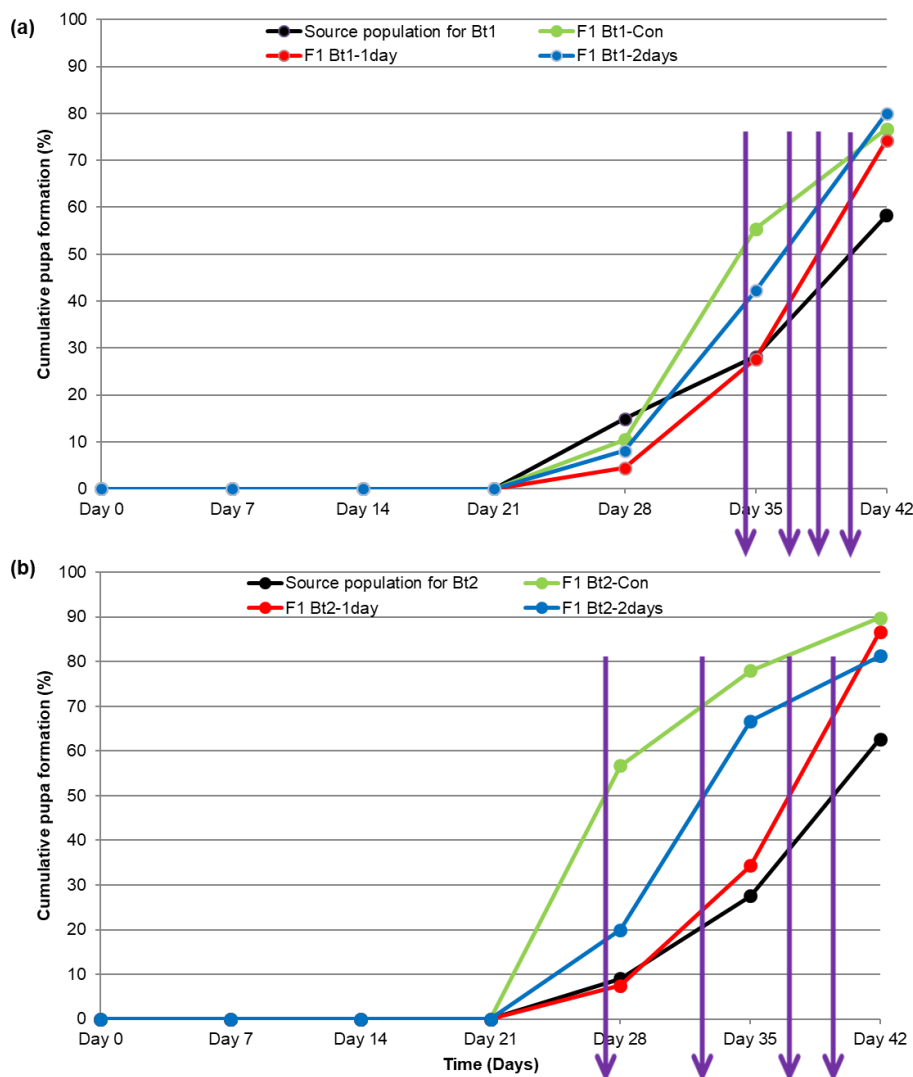


Figure 3-6: The cumulative percentage pupa formation over 42 days for *Chilo partellus* larvae that fed on Bt maize and non-Bt maize (control). Black lines indicate the F0 t generation feeding on Bt maize for 1 day before transfer to non-Bt maize. Green lines indicate F1 generation larvae of the source population feeding on non-Bt maize. Red lines indicate F1 generation larvae of the source population feeding on Bt for 1 day. Blue lines indicate F1 generation larvae of the source population feeding on Bt for 2 days: (a) Bt1 maize; (b) Bt2 maize. Arrows indicate the numbers of days that larvae of each of the generations took until 50% pupa formation.

Table 3-6: The DT₅₀ (days) values for *Chilo partellus* larval development time to pupae after larvae were fed with Bt and non-Bt maize for one or two days before they were transferred to non-Bt maize to complete their life cycles under laboratory conditions

Pupae	DT ₅₀ (days to pupa formation)	Chi-square Goodness of fit	p-value
Bt1 maize			
F0 population Bt1	40.14 (38.39- 42.45)*	6.308	0.177
F1-Bt1-Con	35.77 (31.55- 44.45)	15.182	0.004
F1-Bt1-1day	39.72 (34.05- 63.40)	25.178	0.000
F1-Bt1-2days	37.05 (32.33- 49.27)	23.968	0.000
Bt2 maize			
F0 population Bt2	40.44 (37.92- 44.40)	3.860	0.425
F1-Bt2-Con	29.50 (25.51-33.48)	23.169	0.000
F1-Bt2-1day	37.05 (31.03- 61.91)	11.121	0.025
F1-Bt2-2days	33.37 (30.84- 36.28)	1.141	0.888

*Values in brackets indicate 95% fiducial limits

3.5 Discussion

Larval development

Larvae that were exposed to the Cry1Ab protein for one day during both the first (F0) and second (F1) generations and the F1 larvae which was exposed to Cry1Ab protein for 2 days had a higher survival than that recorded by Tende *et al.* (2005). The larvae that were exposed to Bt2 (Cry1A.105 + Cry2Ab2 proteins) maize tissue had approximately the same percentage survival as that reported by Tende *et al.* (2005) for *C. partellus* on Event 223 which expresses Cry1Ab protein. Tende *et al.* (2010) repeated the same study with more selection cycles and found that fewer larvae survived on Event 223 even after 8 successive cycles. In this study higher survival percentages for Bt1 maize tissue were recorded after only 2 generations and with only a 1 and 2 day exposure period before transfer of larvae onto non-Bt maize tissue. This indicates that *C. partellus* may have developed a slight level of tolerance to the Cry toxins expressed by Bt maize used in this study.

Pupa development

Tende *et al.* (2010) found that the pupae of *C. partellus* larvae that fed on Bt maize weighed less than those that fed on non-Bt maize. However, in this study female pupae of larvae that fed on Bt maize had a higher mass than the first generation exposed to Bt maize and the offspring which developed on non-Bt. When pupal mass is comparatively lower after larvae fed for a short period on Bt, it could indicate that Bt may have an effect on its development. However from these

observations the female mass increased the longer the larvae fed on Bt tissue. This means that the Bt did not influence the ability of larvae to continue feeding on non-Bt tissue. The higher female pupal mass recorded for the F1 pupae, may indicate that there is some level of tolerance to the Bt protein. There was an increase in the larval development rate as well on Bt maize with the F1 larvae which fed on especially Bt2 plant tissue, growing faster and developing into pupae quicker than those of the F0 generation.

3.6 Conclusion

This study shows that it may be possible to select for resistance to Bt toxins in *C. partellus* populations under laboratory conditions. Development time to pupa formation was quicker in the F1 generation than the F0 generation and female pupal mass, which is an indicator of female fecundity, was also higher in the F1 than the F0 generation. These results should however be seen as preliminary and it can only be confirmed after more studies and also more selection cycles.

3.7 References

- Crespo, A.L.B., Rodrigo-Simon, A., Siqueira, H.A.A., Pereira, E.J.G., Ferre, J. & Siegfried, B.D. 2011. Cross-resistance and mechanism of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrina nubilalis*. *Journal of Invertebrate Pathology*, 107: 185-192.
- Dhurua, S. & Gujar, G.T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science*, 67:898-903.
- Farias, J.R., Andow, D.A., Horikoshi, R.J., Sorgatto, R.J., Fresia, P., dos Santos, A.C. & Omoto, C. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. *Crop Protection*, 64: 150-158.
- James, C. 2016. Global Status of Commercialized Biotech/GM Crops: 2016. *ISAAA Brief No. 52*. ISAAA. Ithaca, NY.
- Janmaat, A.F., Wang, P., Kain, W., Zhao, J.Z. & Myers, J. 2014. Inheritance of resistance to *Bacillus thuringiensis* subsp. *kurstaki* in *Trichoplusia ni*. *Applied and Environmental Microbiology*, 70: 5859-5867.
- Kfir, R., Overholt, W.A., Khan, Z.R. & Polaszek, A. 2002. Biology and Management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology*, 47: 701- 731.

Tabashnik, B.E., Cushing, N.L., Finson, N. & Johnson, M.W. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 83: 1671-1676.

Tende, R.M., Nderitu, J.H., Mugo, S., Songa, J.M., Olubayo, F. & Bergvinson, D. 2005. Screening for development of resistance by the spotted stemborer *Chilo partellus* (Lepidoptera: Pyralidae) to Bt-maize delta-endotoxins. *African Crop Science Conference Proceedings*, 7: 1241-1244.

Tende, R.M., Mugo, S.N., Nderitu, J.H., Olubayo, F.M., Songa, J.M. & Bergvinson, D. 2010. Evaluation of *Chilo partellus* and *Busseola fusca* susceptibility to δ -endotoxins in *Bacillus thuringiensis* maize. *Crop Protection*, 29: 115-120.

Tabashnik, B.E., Brévault, T. & Carrière, Y. 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31: 510-521.

Van Rensburg, J.B.J. 1999. Evaluation of Bt.-Transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant and Soil*, 16:38-43.

Van Rensburg, J.B.J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil*, 24: 147-151.

CHAPTER 4

THE PREFERENCE OF *CHILO PARTELLUS* MOTHS AND LARVAE FOR BT AND NON BT MAIZE.

4.1 Abstract

Maize is an important food resource for humans and their livestock. Pests such as *Busseola fusca* and *Chilo partellus* pose a serious threat to maize production in South Africa since borer damage not only results in lower crop yield, but also contributes to ear diseases such as ear rots which enter maize ears through stem borer damage lesions. Bt maize was developed to target such stem borers and improve quality and reduce insecticide use. However, during the nine years after Bt maize with the single gene was commercialized in South Africa, *B. fusca* evolved resistance to it, with the 1st report of resistance made during 2006. To reduce the likelihood of this happening further in the future, new strategies were implemented to delay resistance evolution. An example of insect resistance management strategies that are also being considered is the seed mixture strategy in which Bt and non-Bt maize seed are mixed within the field, creating a mosaic of Bt and non-Bt plants. It has however been reported that larval migration, which could result from non-preference of larvae for plants may lead to sub-lethal exposure to Bt toxin and increased selection for resistance. In this study larval choice tests and moth preference tests were done with *C. partellus* to determine if there is a selective preference for Bt or non-Bt maize. Results indicated that *C. partellus* larvae preferred to feed on leaf tissue of the stacked gene variety and non-Bt maize over the single gene Bt1 maize variety and that the moths did not exhibit preference for either Bt or non-Bt maize plants. Thus, if larvae migrate off Bt maize plants, and they end up on non-Bt maize plants, selection for resistance may be strong.

Keywords: Bt maize, *Chilo partellus*, choice tests, oviposition, preference

4.2 Introduction

Maize is an important food resource not only for humans (usually white maize) but especially for farmers as feed (usually yellow maize) for their livestock (James, 2016). Abiotic factors such as drought are not the only factor that leads to low yields of maize. Biotic factors such as insect pests play also an economically important role in low production of maize. In South Africa there are two economically important stem borer pests of maize i.e. *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Crambidae) (Kfir, 1997). In the past farmers would apply insecticides to their crops since this was the only available method to control stem borer infestations. Brookes and Barfoot (2017) stated that the use of insecticides is not always effective since stem borers in maize feed in hard to reach places where the sprays cannot enter into, for example the stems. Timing of applications is also an important factor when controlling stem borers. Van den Berg and Van Rensburg (1991) found that late single applications of insecticides during the pre-flowering stage of maize are more effective than early single applications since it controls the larger larvae. Since the late 1990's, farmers plant genetically modified Bt maize that targets lepidopteran pests. The adoption of Bt maize in South Africa resulted in a decline of 1.8 million kilograms of active ingredient from 1996 to 2015. These figures therefore indicate a decline of 68.1% in insecticide use on maize over the past 19 years (Brookes & Barfoot, 2017). However, insects are able to evolve resistance to insecticides and therefore also to Bt maize that have insecticidal traits, thus raising concern about resistance evolution. In South Africa during 2006 the first field-evolved resistance to the MON810 event was reported for *B. fusca* in the Vaalharts irrigation scheme area (Van Rensburg, 2007).

Strategies have been implemented to delay resistance development, for example the high dose/refuge strategy (Tabashnik *et al.*, 2003). Refuges are non-Bt host plants planted along with Bt crops to assist in the survival of susceptible pest individuals so that resistant adults can mate with susceptible adults to delay resistance development (Tabashnik *et al.* 2003). Several factors such as behaviour, life history traits and lack of fitness costs contribute to resistance development. Behavioural avoidance or resistance occurs when a resistant insect is able to detect or avoid a toxin (Nansen *et al.*, 2016). An example thereof is when an insect stops feeding when it comes into contact with toxin or it may move to an area or plant material which are not affected by toxin (Nansen *et al.*, 2016). Insects may develop behavioural resistance due to sublethal effects that Bt toxin may have as well as the learning ability and behaviour exhibited by such individuals (Nansen *et al.*, 2016). Behavioural avoidance and strong preference for Bt maize may contribute to resistance development.

The aim of this study was to determine whether *C. partellus* larvae are able to discriminate between non-Bt and Bt maize and if moths exhibited preference for Bt maize over non-Bt maize.

4.3 Materials and methods

Chilo partellus larvae and pupae were obtained from a Potchefstroom population that is maintained at the ARC-GCI in Potchefstroom. The population was kept at 26°C and 50% r.h. with a natural day length exposure of 14L: 10D. The population was reared on artificial diet and the neonate larvae and moths were used in the following experiments.

4.3.1 Preferences of inexperienced neonate larvae

Bioassays were conducted with neonate larvae of *C. partellus*. One set of bioassays was done using small sections (discs) cut from the tightly rolled furl leaves of maize whorl tissues (Fig. 4-1. a), while in the other set of bioassays, larger square cuttings of maize furl leaf tissue were used (Fig. 4-1.b). No-choice, 2-choice and multiple-choice tests were conducted. The maize varieties used in these choice tests were non-Bt, and two Bt maize events referred to as Bt1 (containing the Cry1Ab gene) and Bt2 (containing the Cry1A.105 and Cry2Ab2 genes).

Preferences of larvae were determined on the basis of the numbers of larvae that responded to the respective treatments after a certain period of time. Three experiments were therefore conducted for each of the no-choice, 2-choice and multiple-choice evaluation. Two of these experiments were aimed at assessing larval orientation preference (numbers responding over a period of either 1 or 4 hrs) and one at evaluating larval settling (response after 24 hrs). According to Leiner and Spafford (2016) to determine the settlement preference for larvae the position of the larvae after 24 hr in the arena must be recorded. To determine the orientation preference of larvae their position after 1 h in the choice arena must be recorded. However, if the larvae was not present on one of the treatments after 1 h then it was recorded as a “no-choice” and was not included in the analyses of larval orientation preference (Leiner & Spafford, 2016).

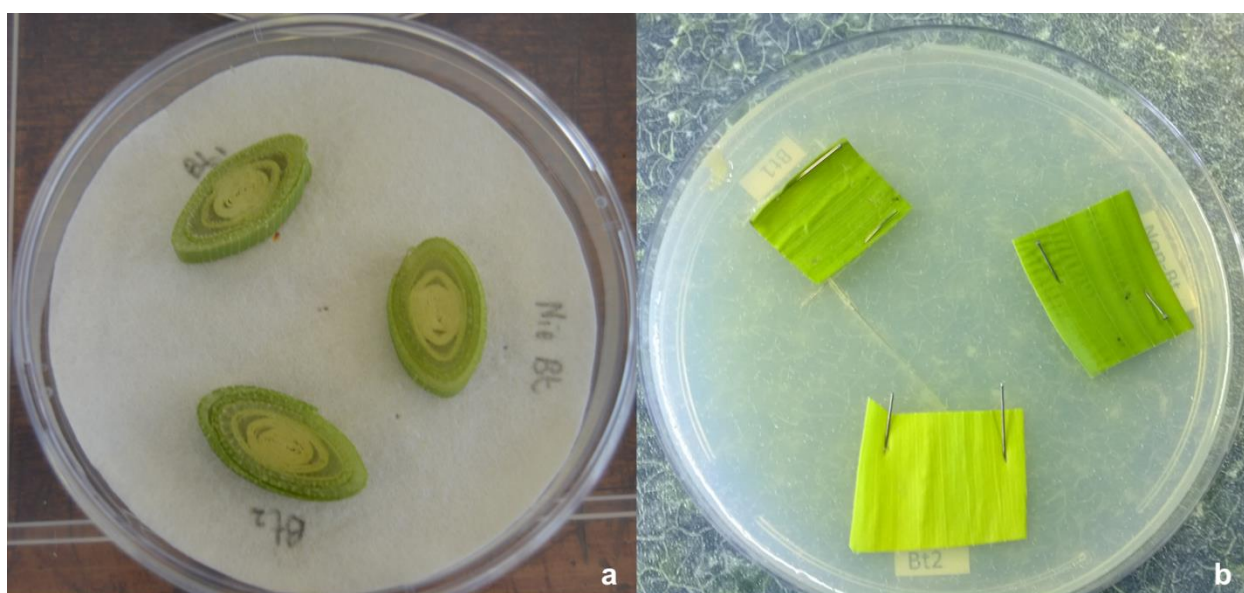


Figure 4-1: A photo illustrating the two different multiple-choice test setups with one petri dish containing: small circular leaf sections (a) and large square cuttings of furl leaves (b)

Small circular leaf discs

The base of maize whorl leaf material was cut into 5 mm maize whorl discs which were then placed at an equidistance of 1.5 cm on filter paper which was put on the bottom of the dishes. Each filter paper disc was moistened with 5 drops of distilled water. Five neonate larvae were put in the centre of each petri dish and left to choose between the small circular leaf discs of different maize plants, provided in different combinations of non-Bt, Bt1 and Bt2 maize leaf discs treatments. The different choice tests are described below.

No-choice test

This test consisted of two types of choice arenas (Fig. 4-2). Five neonate larvae were then put into the centre of the petri dish and their movement monitored. In the other no-choice test, the leaf disc was placed in the centre of the petri dish and five neonate larvae were put on top of the leaf disc after which their movement was monitored. These choice tests were replicated 48 times for all three treatments and the position of larvae in relation to leaf discs recorded at intervals of 1, 4 and 24 hours.

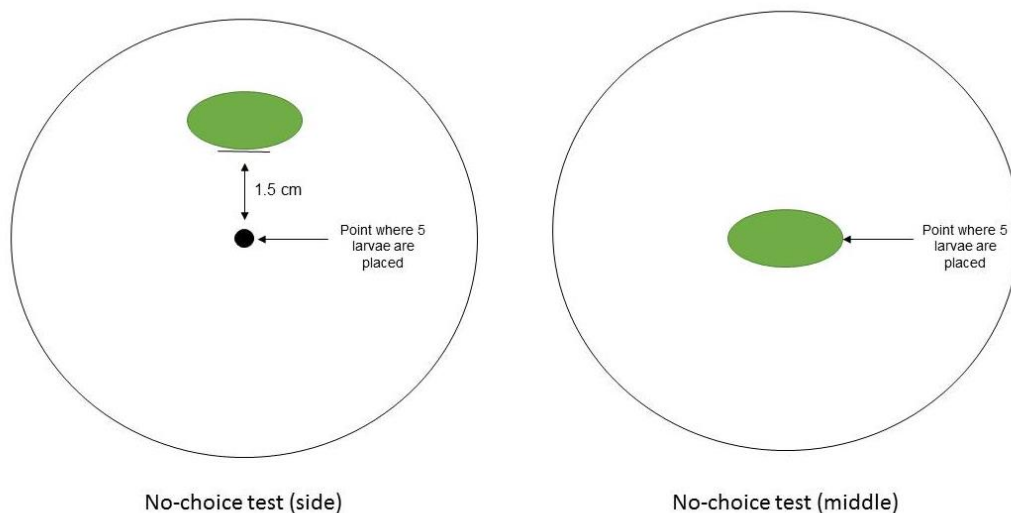


Figure 4-2: A diagram illustrating the layout of the two different no-choice test setups with one petri dish containing a small circular leaf discs on the side (left) and the other containing a small circular leaf discs in the centre (right).

Two-choice tests

This test consisted of a choice arena that contained a combination of two of the treatments as mentioned above. The treatments were placed from the centre and five neonate larvae were

placed in the centre of the petri dish and left to choose between the treatments. Observations were made at similar time intervals as described for the no-choice tests. This experiment was replicated 48 times for each treatment combination (Fig. 4-3).

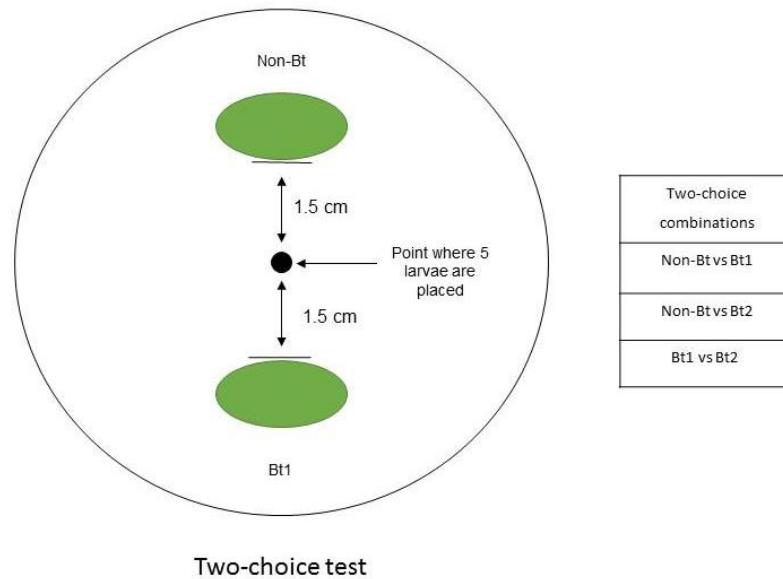


Figure 4-3: A diagram illustrating the layout of a two-choice test that has a choice arena containing non-Bt and Bt1 small circular leaf discs, and the different treatment combinations.

Multiple-choice tests

This test consisted of a combination of non-Bt, Bt1 and Bt2 leaf tissue in the same choice arena (Fig 4-4). Leaf discs were placed at an equidistance from each other and from the centre. Five neonate larvae were then placed in the centre of the petri dish. Observations were made at similar intervals as described above. This experiment was replicated 48 times.

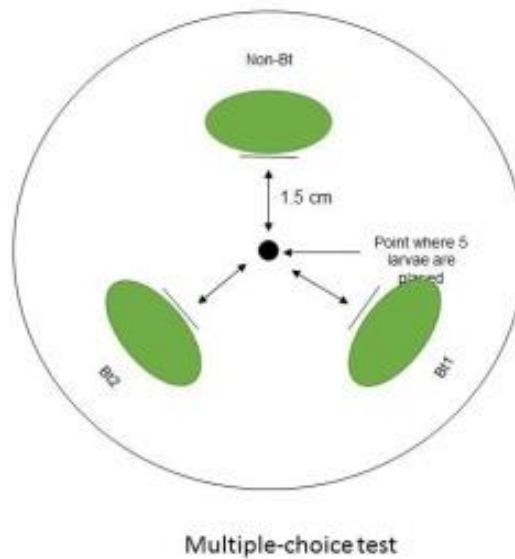


Figure 4-4: A diagram illustrating the layout of the three treatments non-Bt, Bt1 and Bt2 in the choice arena.

Large cuttings of maize leaf tissue

Large cuttings of maize leaf tissues (1.5 cm in width and 3 cm long) were made from the inner whorl leaves of maize plants. The leaves were then folded in two and pinned onto agar plates (agar plates were prepared the day before the experiment). The purpose of the pins was to hold the leaf tissue flat to the surface of the agar to prevent folding and also allows for favourable thigmotaxic conditions for the larvae. The same choice combinations used in the assays with the small circular leaf discs were used in this experiment (Fig. 4-5). The petri dishes were left for the same time intervals as mentioned above and the number of larvae that were recorded underneath or on the leaf was counted. This experiment was replicated 48 times.

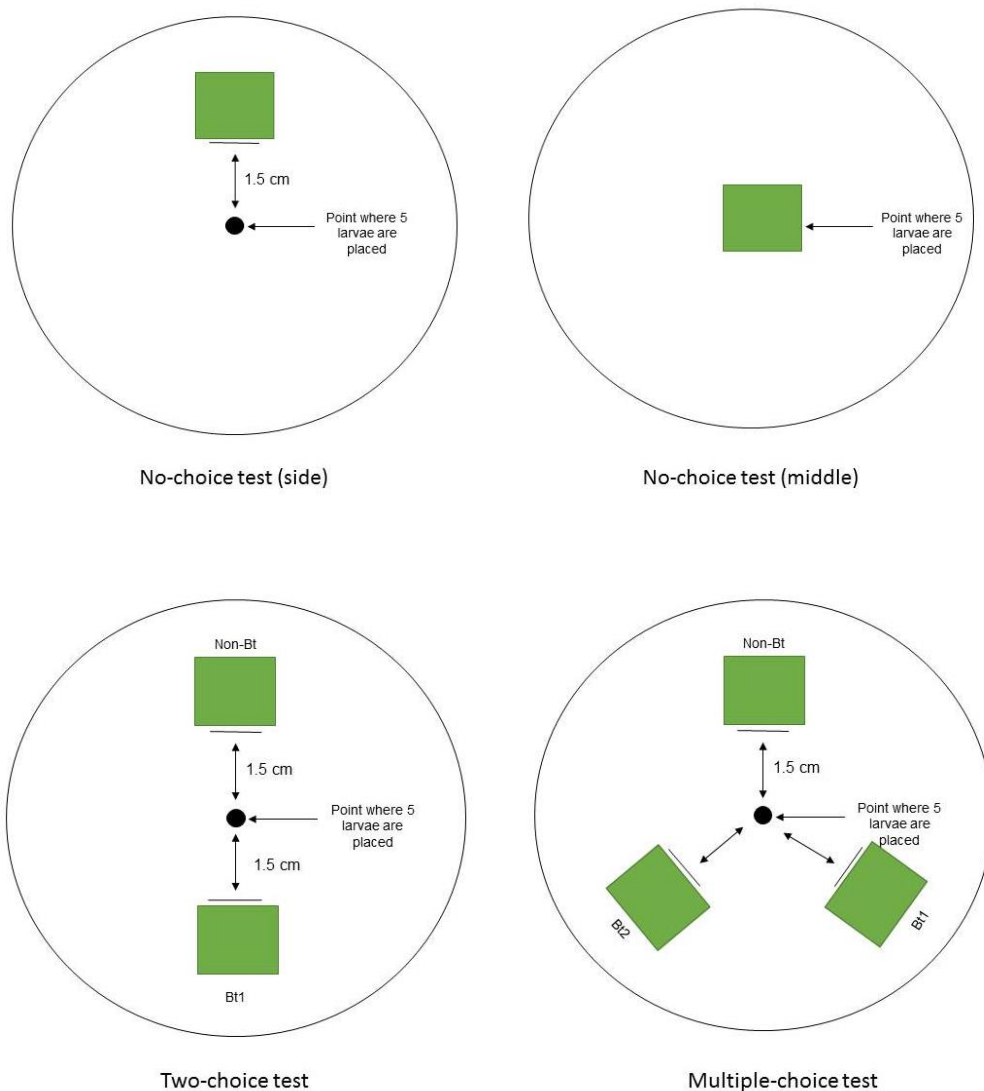


Figure 4-5: A diagram illustrating the layout of the four types of choice combinations used in the bioassays with large pieces of maize whorl leaf tissue and inexperienced neonate larvae.

4.3.2 Preference of experienced neonate larvae

An experiment was done to determine if larval orientation and settlement preferences are influenced after larvae fed on leaf tissue of the different leaf treatments prior to providing them with a choice between leaf tissue of different maize treatments. Neonate larvae were provided with leaf tissue of the different maize treatments in order for them to experience feeding on the particular maize treatment. This was done by allowing them to feed for different time periods before transferring them into petri dishes where their responses to different maize treatments was evaluated in a multiple-choice setup. Leaf tissue provided for larvae during the preconditioning period as well as during multiple-choice experiment, was provided in the form of leaf cutting (1.5 cm in width and 3 cm long). These cuttings were obtained from the inner whorl of maize plants from each treatment. These leaves were then folded and pinned onto filter paper in the centre of

a petri dish. For the preconditioning phase, larvae were put onto each of the leaf cuttings while larvae were put in the centre of the petri dish in the multiple-choice experiment that followed.

This preconditioning (experience) feeding period lasted for either 1, 4 or 24 hours on the different maize treatments to precondition them to a particular maize treatment (Fig. 4-6). Larval orientation (short-term response) was evaluated after 1 hour for the larvae that were preconditioned for 1 hr, after 4 hour for larvae that were preconditioned for 4 hours and 24 hours for. The experiment was replicated 48 times.

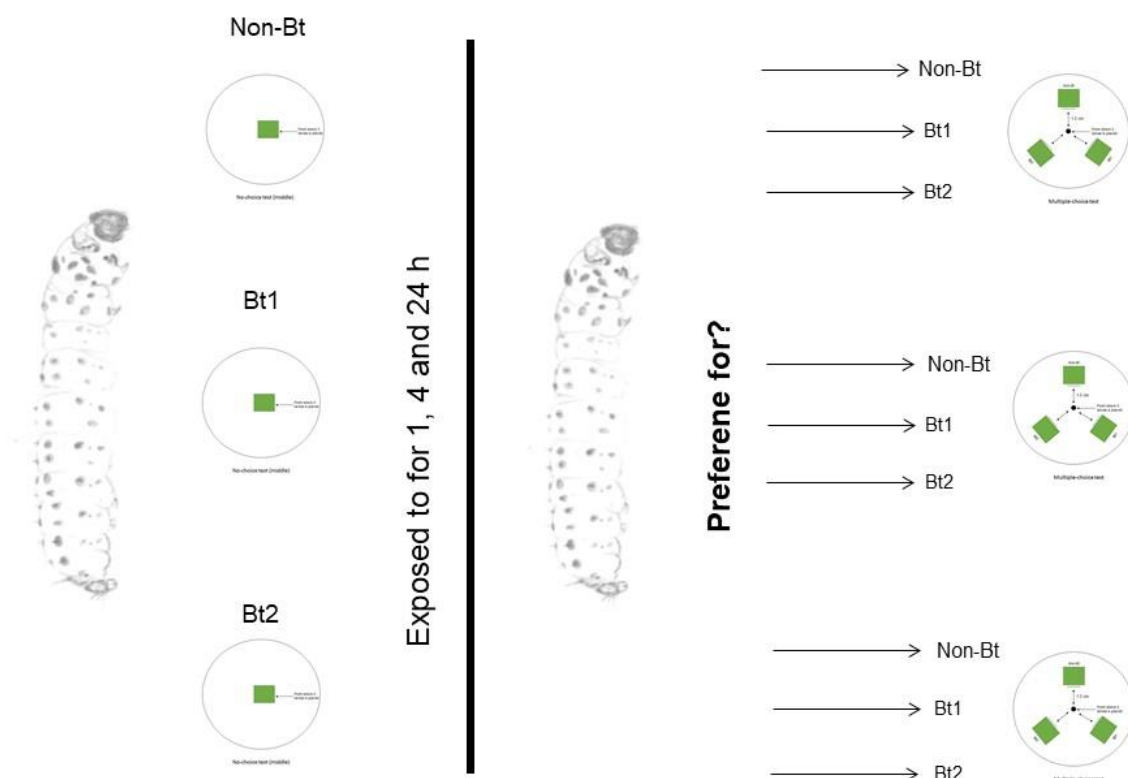


Figure 4-6: Schematic representation of the choice test done with *Chilo partellus* larvae that were exposed to the three treatments before transfer into a multi-choice arena.

4.3.3 Oviposition preference

Oviposition preference of moths

The experiments were conducted in a greenhouse at the Agricultural Research Council-Grain Crops Institute in Potchefstroom. Oviposition cages measuring 37 cm in length and 23 cm in diameter with wire mesh wall covering were used (Fig. 4-7). The top of the cage was covered with

nets, held in place by rubber bands. The cages were inserted into the upper rims of the pots (Fig. 4-6). The pots were planted with a two-choice combination of either non-Bt, Bt1 or Bt2 maize.

One male and one female moth that emerged on the same day were placed into each cage. For example, in each cage a combination of one non-Bt and one Bt2 was placed (2 plants/pot). The plants were used in the experiment when they reached a height of 30 cm (5 leaves/ 3-5 weeks), which is the preferred plant growth stage for natural infestation of maize by *C. partellus* (Bate and Van Rensburg, 1992). After five days, the stems of plants were cut off at soil level and the number of egg batches and eggs per plant was counted. This experiment was replicated 15 times. Experiments were done at a temperature which ranged between 19 and 25 °C and a light: dark period of 14L:10D.



Figure 4-7: Photo illustrating the set-up of the oviposition choice experiments.

Data analysis

Data on larval responses to various treatments were expressed as percentages. Data on the choices of inexperienced larvae, were analysed by means of a Student's t-test for the two-choice data and a ANOVA for the no-choice and multiple-choice data. Data on responses of experienced larvae were analysed by means of an ANOVA using Genstat edition 18. The data on moth responses to the various treatments were analysed by means of a Student's t-test.

4.4 Results

4.4.1 Preferences of inexperienced neonate larvae

Bioassays with small circular leaf discs

No-choice test

There were no significant differences between the three treatments when neonate larvae were left to feed for 1 hour as well as 4 hours (Table 4-1). However, there were significantly more larvae on the non-Bt leaf discs than on the two Bt treatments leaf discs after 24 hours. There were however still significant numbers of larvae left on the leaf discs. This tendency was not observed for the larvae that were inoculated onto the filter paper (Table 4-2). More larvae were present on the leaf discs after 24 hours, but a few larvae climbed off Bt2 leaf discs after 24 hours.

Two-choice test

The numbers of larvae that were given the choice between a leaf disc of non-Bt and Bt1 did not differ significantly between treatments 1, 4 and 24 hours after inoculation, although there was a tendency of higher numbers being present on non-Bt than Bt1 (Table 4-3). The same tendency was observed for the two-choice combination for non-Bt and Bt2, but more larvae preferred Bt2 instead of non-Bt. After 24 hours significantly higher numbers of larvae were present on non-Bt than Bt2 tissue. When given the choice between Bt1 and Bt2 circular leaf discs, larvae preferred Bt2 over Bt1 after the first hour and again after 24 hours.

Multiple-choice test

When inexperienced neonate larvae were given a choice between the three treatments, there were no significant differences in their numbers per disc of the respective treatments over the first 4 hours. After 24 hours significantly fewer larvae (19.58%) were present on the Bt1 discs compared to non-Bt and Bt2 leaf discs (Table 4-4).

Table 4-1: Mean percentage of *Chilo partellus* larvae after one, four and 24 hours after inoculation in a no-choice test with small circular leaf discs put in the centre of petri dishes.

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	100.00 a*		99.58 a		95.42 b	
Bt1	99.58 a		99.17 a		83.75 a	
Bt2	96.67 a		99.58 a		82.92 a	
	F-value	p-value	F-value	p-value	F-value	p-value
	3.98	0.058	0.30	0.748	12.60	0.002

* Means within columns followed by a different letter, differ significantly ($p < 0.05$)

Table 4-2: Mean percentage of *Chilo partellus* larvae after one, four and 24 hours after inoculation in a no-choice test with small circular leaf discs at the side

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	78.75 a*		82.50 a		94.58 a	
Bt1	83.75 a		85.42 a		97.92 a	
Bt2	80.42 a		88.33 a		87.50 a	
	F-value	p-value	F-value	p-value	F-value	p-value
	0.11	0.897	0.88	0.449	1.23	0.337

* Means within columns followed by a different letter, differ significantly ($p < 0.05$)

Table 4-3: Mean percentage of *Chilo partellus* larvae on small circular leaf discs after one, four and 24 hours after inoculation a in two-choice bioassay.

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	47.92		43.33		55.00	
Bt1	36.67		45.83		38.33	
	t-value	p-value	t-value	p-value	t-value	p-value
	-2.50	0.014	-0.56	0.577	2.93	0.004
Non-Bt	36.67		40.00		48.33	
Bt2	48.75		50.42		38.33	
	t-value	p-value	t-value	p-value	t-value	p-value
	2.53	0.013	-2.09	0.039	2.09	0.039
Bt1	42.50 a		40.42		35.42	
Bt2	49.17 a		50.42		55.00	
	t-value	p-value	t-value	p-value	t-value	p-value
	-1.34	0.183	-2.01	0.047	-4.92	< 0.001

Table 4-4: Mean percentage of *Chilo partellus* larvae on small circular leaf discs after one, four and 24 hours after inoculation for a multiple choice test.

	Orientation (1Hour)		Orientation (4Hour)		Settling (24Hour)	
Non-Bt	28.33 a*		27.92 a		39.58 b	
Bt1	29.17 a		31.25 a		19.58 a	
Bt2	33.33 a		33.33 a		34.58 ab	
	F-value	p-value	F-value	p-value	F-value	p-value
	0.30	0.749	0.29	0.756	5.57	0.027

*Means within columns followed by a different letter differ significantly ($p < 0.05$).

Bioassays with larger square cuttings of maize leaf tissue.

No-choice tests

There were no significant differences between the three treatments after larvae fed for 1, 4 and 24 hours after inoculation on top of the leaf. However, there were less larvae on top the two Bt treatments after 24 hours (Table 4-5). The same tendency was observed for the leaf tissue that was placed at the side (Table 4-6). However, after 24 hours there were more larvae on top of the three treatments.

Two-choice tests

Neonate larvae that were given a choice between two leaf tissue treatments are indicated in Table 4-7. The percentage of larvae on non-Bt and Bt1 maize leaf discs, did not differ significantly after 24 hours. Although there was a tendency that a higher percentage of larvae occurred on more Bt2 leaf discs (50.00%) compared to non-Bt (38.75%) after 4 hours, more larvae chose significantly more non-Bt over Bt2 after 24 hours. In the Bt1/Bt2 two-choice combination there were no significant differences between the two treatments after 4 and 24 hours.

Multiple-choice tests

When larvae were given the choice between the three different treatments, there were no significant differences in larval response between after 1 or 24 hours after inoculation (Table 4-8). However, at 4 hours after inoculation more larvae selected Bt2 tissue (35.00%) than Bt1 (22.08%). After 24 hours more larvae chose Bt2 tissue than that of the other two treatments although no significant differences were observed.

Table 4-5: Mean percentage of *Chilo partellus* larvae after one, four and 24 hours after inoculation in a no-choice test with large square cuttings of maize leaf tissue in the centre.

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	72.50 a*		94.17 a		89.58 a	
Bt1	88.33 a		87.50 a		80.83 a	
Bt2	90.42 a		93.75 a		82.08 a	
	F-value	p-value	F-value	p-value	F-value	p-value
	2.05	0.184	2.24	0.163	2.37	0.149

* Means within columns followed by a different letter, differ significantly ($p < 0.05$)

Table 4-6: Mean percentage of *Chilo partellus* larvae after one, four and 24 hours after inoculation in no-choice test with large square cuttings of maize leaf tissue at the side.

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	71.25 a*		81.67 a		87.50 a	
Bt1	79.17 a		80.83 a		80.00 a	
Bt2	69.58 a		71.25 a		76.25 a	
	F-value	p-value	F-value	p-value	F-value	p-value
	1.38	0.300	2.17	0.170	1.16	0.357

* Means within columns followed by a different letter, differ significantly ($p < 0.05$)

Table 4-7: Mean percentage of *Chilo partellus* larvae on large square cuttings of maize leaf tissues of different maize treatments after one, four and 24 hours after inoculation in two-choice tests.

	Orientation (1 hour)		Orientation (4 hours)		Settling (24 hours)	
Non-Bt	36.67		48.75		43.75	
Bt1	49.58		36.67		41.25	
	t-value	p-value	t-value	p-value	t-value	p-value
	-2.13	0.035	2.40	0.018	0.45	0.656
Non-Bt	43.75		38.75		58.33	
Bt2	49.58		50.00		30.00	
	t-value	p-value	t-value	p-value	t-value	p-value
	-1.06	0.290	-2.06	0.043	4.79	< 0.001
Bt1	51.25		42.50		44.58	
Bt2	27.50		40.00		37.50	
	t-value	p-value	t-value	p-value	t-value	p-value
	4.12	< 0.001	0.50	0.616	1.37	0.172

Table 4-8: Mean percentage of *Chilo partellus* larvae on large square cuttings of maize leaf tissues for one, four and 24 hours after inoculation for a multiple-choice test.

	Orientation (1Hour)		Orientation (4Hour)		Settling (24Hour)	
Non-Bt	26.67 a*		33.75 ab		25.00 a	
Bt1	35.83 a		22.08 a		30.00 a	
Bt2	28.75 a		35.00 b		34.58 a	
	F-value	p-value	F-value	p-value	F-value	p-value
	1.31	0.316	4.78	0.038	0.51	0.616

*Means within columns followed by a different letter differ significantly ($p < 0.05$).

4.4.2 Preference of experienced neonate larvae

Larvae that fed on non-Bt tissue prior to choice tests

Larvae that were preconditioned for one hour and then given a choice between the three treatments chose non-Bt leaf tissue over Bt2 (Table 4-9). However, when the larvae were preconditioned for four and 24 hours respectively and then given the choice between the three treatments there were no significant differences observed.

Larvae that fed on Bt1 tissue prior to choice tests

Larvae that were preconditioned for one hour and then given a choice between the three treatments chose non-Bt over Bt1. The same tendency for four and 24 hours were observed as the larvae that were preconditioned on non-Bt leaf tissue.

Larvae that fed on Bt2 tissue prior to choice tests

Larvae that were fed with Bt2 maize leaf tissue were also given a choice between the three treatments. There were no significant differences in larval orientation and settling on any of the three treatments at any time interval.

Table 4-9: The mean percentage of *Chilo partellus* larvae on large cuttings of maize leaf tissue after one, four and 24 hours of inoculation, after previous feeding on the same three treatments before transfer into the multiple-choice test.

Before → After*	Orientation (1Hour)		Orientation (4 Hour)		Settling (24 Hour)	
Non-Bt → Non-Bt	43.06 b**		34.97 a		26.94 a	
Non-Bt → Bt1	30.45 ab		32.26 a		23.30 a	
Non-Bt → Bt2	21.88 a		28.65 a		28.37 a	
	F- value	p-value	F- value	p-value	F- value	p-value
	10.38	0.005	0.83	0.469	0.41	0.673
Bt1 → Non-Bt	40.76 b		30.56 a		19.17 a	
Bt1 → Bt1	24.13 a		24.17 a		10.49 a	
Bt1 → Bt2	29.06 ab		36.53 a		13.99 a	
	F- value	p-value	F- value	p-value	F- value	p-value
	5.22	0.031	2.05	0.185	1.27	0.328
Bt2 → Non-Bt	34.79 a		30.66 a		38.85 a	
Bt2 → Bt1	21.94 a		26.62 a		29.13 a	
Bt2 → Bt2	30.80 a		28.44 a		18.30 a	
	F- value	p-value	F- value	p-value	F- value	p-value
	2.92	0.105	0.48	0.631	3.22	0.088

*Larvae were exposed to non-Bt, Bt1 and Bt2 before being transferred to new petri dish with the three combinations.

** Means within columns followed by a different letter differ significantly (p<0.05).

4.4.3 Oviposition preference

Female moths were provided with a choice between two maize plant treatments. For the control treatment where 2 non-Bt plants were provided to choose from, no significant differences were

observed between either the number of egg batches per plant, number of eggs laid per plant and the number of eggs per batch (Table 4-10). For the non-Bt/ Bt1 combination no significant differences in the number of eggs and number of egg batches per plant were observed. No significant differences were observed between number of eggs that were laid per batch on the non-Bt and Bt1 treatments. However, in the non-Bt and Bt2 combination there was a significant difference between the mean number of egg batches per plant of the two treatments, with more egg batches on non-Bt than Bt2 plants. There were no significant differences between the number of eggs per plant and eggs per egg batch on non-Bt and Bt2 plants. In the two choice combination with Bt1 and Bt2 plants, there were no significant differences between any of the parameters.

Table 4-10: The mean number of egg batches per plan, eggs per plant and eggs per batch, laid by *Chilo partellus* in two-choice tests.

Choice conditions	Mean number of egg batches per plant	Mean number of eggs per plant	Mean number of eggs per batch
Non-Bt maize	2.80	54.27	19.38
Non-Bt maize	2.67	54.53	20.45
t-value	0.13	-0.01	-0.24
p-value	0.900	0.990	0.812
Non-Bt maize	2.47	51.87	21.03
Bt1 maize	2.87	69.53	26.14
t-value	-0.45	-0.91	-0.91
p-value	0.656	0.373	0.368
Non-Bt maize	2.53	68.93	27.21
Bt2 maize	0.87	28.13	32.46
t-value	2.27	1.77	-0.59
p-value	0.038	0.091	0.556
Bt1 maize	2.67	65.80	24.68
Bt2 maize	2.13	59.40	27.84
t-value	0.680	0.260	-0.57
p-value	0.505	0.796	0.569

4.5 Discussion

Preferences of inexperienced neonate larvae

When neonate larvae were provided one choice of treatment, they preferred to remain on the treatments. In choice tests done with *Trichoplusia ni* (Lepidoptera: Noctuidae) on non-Bt and Bt cotton, Li *et al.* (2006) found that when larvae were given a no-choice test, 100% of the larvae would remain on the leaf, but when presented with a Bt leaf, they would be more active and climb on and off the discs. Similar observations were made in the no-choice experiments done with *C. partellus* in this study.

However, when circular maize leaf discs were provided in a two choice set-up, more of the larvae preferred non-Bt maize over Bt1 and Bt2. When square cuttings of maize leaf tissue were provided, more larvae chose Bt1 over non-Bt and Bt2. Li *et al.* (2006) also found that when larvae of *T. ni* were given a choice between non-Bt and Bt cotton leaves, larvae preferred the non-Bt leaves. However in this study, *C. partellus* larvae did not show differences in orientation and

settling on non-Bt and Bt2 tissue, which was reported by Li *et al.* (2006) for Bt cotton leaves which also expressed the Cry2Ab2 Bt protein.

When larvae were given a choice between the three treatments, larvae would both prefer non-Bt and Bt2. This may be due to both treatments having the same chemical cues.

Preference of experienced neonate larvae

When larvae were given a choice between three treatments after feeding on non-Bt leaf tissue, they initially orientated towards non-Bt tissue but after a 24-hour period, the majority of larvae settled on Bt2 leaf tissue. In a similar scenario when larvae fed on Bt1 tissue prior to the assay, they would also orientate towards non-Bt and Bt2 tissue instead of Bt1. When larvae fed on Bt2 tissue prior to the multiple choice test, they orientated towards non-Bt and Bt2 tissue. These results could indicate that chemical cues emitted by leaf disc material may initially attract more larvae towards non-Bt and Bt2 tissue. However, after 24 hours more larvae settled on Bt1 than on Bt2 and non-Bt tissue. Reasons for these results are unknown at present.

Oviposition preference

The female moths of *C. partellus* did not show preference for any of the treatments in any of the choice combinations (Table 4-10).

Obonyo *et al.* (2008) found that when female moths of *C. partellus* and *Sesamia calamistis* (Lepidoptera: Noctuidae) were given a choice between non-Bt and Bt maize (Cry1Ab), the moths did not exhibit preference for any of the treatments. This correlates with not only this study but also other related studies on oviposition of moths on non-Bt and Bt plants. A study done by Luong *et al.* (2016) found that the female moths of *Helicoverpa armigera* (Lepidoptera: Noctuidae) did not discriminate between non-Bt and Bt cotton plants when they laid their eggs. Shera and Arora (2015) reported that *Earias vitella* (Fabricius) (Lepidoptera: Noctuidae) also did not discriminate between non-Bt and Bt cotton plants when presented with a choice. However, Tellez-Rodriguez *et al.* (2014) found that *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) moths exhibited oviposition preference for Bt maize in a choice set-up with non-Bt maize. The latter study by Tellez-Rodriguez *et al.* (2014) was however done under field conditions whereas the other studies were done under laboratory conditions.

4.6 Conclusions

Chilo partellus moths did not exhibit any preference for Bt or non-Bt plants. If this also applies under field conditions, the result would be that female moths may be more likely to lay their eggs on Bt maize inside seed mixture plantings, since the abundance of Bt plants in such mixtures is high (>80%). The offspring of these moths will therefore most likely die if they only encounter Bt

plants. If larvae feed on the natal Bt plant tissue for less than 24 hours before it migrates to a non-Bt neighbouring plant, it is likely to survive the initial Bt exposure (Chapter 3), which then exerts selection pressure for resistance evolution. Further studies should be done to investigate the volatile or chemical cues emitted by non-Bt and Bt maize plants and its effect on *C. partellus* larval and moth responses towards such plants.

4.7 References

Bate, R. & Van Rensburg, J.B.J. 1992. Predictive estimation of maize yield loss caused by *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in maize. *South African Journal of Plant and Soil*, 9: 150-154.

Brookes, G. & Barfoot, P. 2017. Environmental impacts of genetically modified (GM) crop use 1996-2015: impacts on pesticide use and carbon emissions. *GM Crops & Food*, 8: 117-147.

James, C. 2016. Global Status of Commercialized Biotech/GM Crops: 2016. *ISAAA Brief No. 52*. ISAAA. Ithaca, NY.

Kfir, R. 1997. Natural control of the cereal stem borers *Busseola fusca* and *Chilo partellus* in South Africa. *Insect Science and its Application*, 17: 61-67.

Kfir, R., Overholt, W.A., Khan, Z.R. & Polaszek, A. 2002. Biology and Management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology*, 47: 701-731.

Leiner, R. & Spafford, H. 2016. Pickleworm (*Diaphania nitidalis* Cramer) neonate feeding preferences and the implications for a push-pull management system. *Insects*, 7: 1-11.

Li, Y.-X., Greenberg, S.M. & Liu, T.-X. 2006. Effects of Bt cotton expressing Cry1Ac and Cry2Ab and non-Bt cotton on behaviour, survival and development of *Trichoplusia ni* (Lepidoptera: Noctuidae). *Crop Protection*, 25: 940-948.

Luong, T.T.A, Downes, S.J., Cribb, B., Perkins, L.E. & Zalucki, M.P. 2016. Oviposition site selection and survival of susceptible and resistant larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Bt and non-Bt cotton. *Bulletin of Entomological Research*, 106: 710-717.

Nansen, C., Baissac, O., Nansen, M., Powis, K. & Baker, G. 2016. Behavioural avoidance-will physiological insecticide resistance level of insect strains affect their oviposition and movement responses? *PLoS ONE* 11(3): e0149994. doi:10.1371/journal.pone.0149994.

Obonyo, D.N., Songa, J.M., Oyieke, F.A., Nyamasyo, G.H.N. & Mugo, S.N. 2008. Bt-transgenic maize does not deter oviposition by two important African cereal stem borers, *Chilo partellus*

Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Journal of Applied Biosciences*, 10: 424-433.

Shera, P.S. & Arora, R. 2015. Comparative study on oviposition and larval preference of spotted bollworm, *Earias vittella* on Bt and non-Bt cotton. *Journal of Environmental Biology*, 37: 121-127.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J. 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. *Journal of Economic Entomology*, 96: 1031-1038.

Téllez-Rodríguez, P., Raymond, B., Moran-Bertot, I, Rodríguez-Cabera, L., Wright, D.J., Borotto, C.G. & Ayra-Pardo, C. 2014. Strong oviposition preference for Bt over non-Bt maize in *Spodoptera frugiperda* and its implications for the evolution of resistance. *BMC Biology*, 12: 1-10.

Van den Berg, J. & Van Rensburg, J.B.J. 1991. Unavoidable losses in insecticidal control of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in maize and grain sorghum. *South African Journal of Plant and Soil*, 8: 12-16.

Van Rensburg, J.B.J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil*, 24: 147-151.

CHAPTER 5

CONCLUSIONS

Prior to, and since the approval of Bt maize for commercial release in South Africa during 1998, a limited number of studies on the response of *Chilo partellus* to Bt maize were done. One such publication on Bt maize was by Van Rensburg (1999) in which he evaluated the level of resistance of *C. partellus* to MON810 Bt maize. A few studies on the response of *C. partellus* to Bt maize, especially in East Africa and India have however been reported, even though Bt maize has not been approved for commercial release in these countries. A list and brief summary of the available literature on interactions between *C. partellus* and Bt Cry proteins in artificial diets as well as Bt maize plants is provided in Table 5-1.

In this study the response of *C. partellus* larvae and moths were tested in different experiments to determine if the adults and the larvae would respond differently to non-Bt and Bt treatments. The two Bt treatments were Bt1 which contained the Cry1Ab gene and Bt2 which is a stacked gene variety that expresses Cry1A.105 and Cry2Ab2 proteins. When neonate larvae of different regions were screened on these two Bt maize events, none of the *C. partellus* larvae survived longer than 11 days on Bt1 (Cry1Ab event) maize (Chapter 2). These results are supported by Mugo *et al.* (2011) who also reported no survival on MON810 hybrids after 10 days when Bt maize plants were infested with neonate *C. partellus* larvae. Singh *et al.* (2005) found similar results when MON810 maize plants were infested with *C. partellus* larvae. This indicates that resistance have not developed. However, all of these studies were done only with neonate larvae and questions arose regarding the fact that no resistance of *C. partellus* to Bt maize in South Africa has been reported after 19 years, while *Busseola fusca* was able to develop resistance to Bt1 maize after only 8 years. Furthermore, it is also important to consider aspects of *C. partellus* behaviour (e.g. larval preference and movement between plants, and moth preferences and behaviour) that may drive selection for resistance in this pest. A suggestion was made that larger larvae that may move from non-Bt plants to Bt plants when they are older, may be subjected to sub-lethal concentrations of Bt toxins, leading to selection for resistance.

Bioassays were conducted during which *C. partellus* larvae fed on non-Bt maize for seven and 16 days respectively before they were transferred to different Bt maize treatments (Chapter 2). This was done to determine if larval age have an effect on survival on Bt maize. *Chilo partellus* larvae that fed for seven days on non-Bt before being transferred to Bt maize died on both types of Bt maize treatments within 14 days. A different result was observed for larvae that fed for 16 days on non-Bt maize before being transferred to Bt maize, with survival being recorded on both Bt events.

A previous study on larval movement of *B. fusca* between non-Bt and Bt plants showed that if they migrated from Bt2 maize plants after 9 days onto non-Bt maize plants, the larvae were able to survive (Erasmus *et al.* 2016), placing strong selection pressure for resistance evolution in the population. Our results indicated that the age of the larvae have an effect on their survival on Bt maize.

An attempt was made during this study to determine the possibility of selecting for resistance in *C. partellus* and to develop a Bt resistant colony (Chapter 3). This was done by allowing larvae to feed on Bt maize tissue for short periods of time (1 or 2 days) before transferring the surviving larvae to non-Bt maize tissue. Higher larval survival was recorded on Bt1 maize tissue than Bt2, but this differs from the findings of Tende *et al.* (2005), who also attempted to select for resistance in a *C. partellus* population. In his study, larvae that fed on Cry1Ab Bt maize had lower survival than that recorded in this study. Our results indicate that it may be possible to select for resistance in *C. partellus* since small increases in larval fitness were observed after one cycle of selection. This also indicates that larval age affects the survival on Bt maize. Our results also show that larvae may have the ability to survive if they move off natal Bt plants onto non-Bt plants within 24 hours of hatching (Chapter 4). *Chilo partellus* larvae and moths were given choices between non-Bt treatments and the two Bt treatments under choice conditions. Female moths did not show preference for any of the two Bt or non-Bt treatments, thus they could not distinguish between non-Bt and Bt maize. If this occurs in the field, female moths of *C. partellus* will most likely lay their eggs on Bt plants, due to the high abundance thereof (<80%) in seed mixture plantings. In a high dose/refuge strategy setup, on the other hand, moths will most likely lay similar numbers of eggs on Bt and non-Bt plants since these plants form part of a maize landscape in which moths do then not exhibit preference for any particular maize variety. If eggs are laid on Bt plants and neonate larvae show non-preference for such plants, they migrate from Bt plants to non-Bt plants, resulting in increased selection for resistance and increased survival of individuals that are heterozygous for resistance. However, if eggs are laid on non-Bt plants and *C. partellus* larvae have a strong non-preference for that particular variety they may move from non-Bt maize to Bt maize plants. Similar results were found by Tellez-Rodriguez *et al.* (2014) with *Spodoptera frugiperda* since these larvae preferred Bt maize over non-Bt if non-Bt maize were already infested with larvae. Results of our study did, however, show no strong preference by *C. partellus* for non-Bt over Bt2 plants, and only slight preference for Bt1 plant tissue. This indicates that larvae most likely cannot distinguish between non-Bt and Bt maize. Further studies should be done on *C. partellus* larval migration and preference for different Bt and non-Bt maize varieties, in order to make informed decisions when insect resistance management strategies are developed.

Table 5-1: A brief summary of studies done on *Chilo partellus* and its response to Bt maize plant material and Bt protein incorporated artificial diet bioassays.

Type of evaluation	Bt protein evaluated	Continent	Source
Bt maize screening	Cry1Ab	Africa	Van Rensburg (1999)
Bt maize screening	Cry1Ac, Cry1B, Cry1E, Cry1B-1Ab, Cry1Ab	Africa	Mugo <i>et al.</i> (2004)
Bt maize effect on beneficial parasitoid	Cry1Ab	Europe	Prütz and Dettner (2004)
Bt maize screening	Cry1Ab	Africa	Tende <i>et al.</i> (2005)
Oviposition on Bt maize	Cry1Ab	Africa	Midega <i>et al.</i> (2006)
Bt maize screening	Cry1Ab	India	Hari <i>et al.</i> (2007)
Oviposition on Bt maize	Cry1Ab	Africa	Obonyo <i>et al.</i> (2008)
Bt maize screening	Cry1Ab	Africa	Obonyo <i>et al.</i> (2008)
Bt maize screening	Cry1Ab	Africa	Tende <i>et al.</i> (2010)
Bt maize screening	Cry1Ab	Africa	Mugo <i>et al.</i> (2011)
Bt maize screening	Cry1Ab	Africa	Murenga <i>et al.</i> (2011)
Bt maize screening	Cry1Ab	Africa	Tefere <i>et al.</i> (2016)

5.1 References

Erasmus, A., Marais, J. & Van den Berg, J. 2016. Movement and survival of *Busseola fusca* (Lepidoptera: Noctuidae) larvae within maize plantings with different ratios of non-Bt and Bt seed. *Pest Management Science*, 72: 2287-2294.

Hari, N., Jindal, J., & Malhi, N. 2007. Resistance of Cry1Ab maize to spotted stemborer *Chilo partellus* (Lepidoptera: Crambidae) in India. *International Journal of Tropical Insect Science*, 27: 223-228.

Midega, C.A.O, Khan, Z.R., Van Den Berg, J., Ogot, C.K.P.O., Pickett, J.A. & Wadhams, L.J. 2006. Maize stemborer predator activity under 'push – pull' system and Bt-maize: A potential component in managing Bt resistance. *International Journal of Pest Management*, 52: 1-10.

Mugo, S., Taracha, C., Bergvinson, D., Odhiambo, B., Songa, J., Hoisington, D., McLean, S., Ngatia, I. & Gethi, M. 2004. Screening cry proteins produced by Bt maize leaves for activity against Kenyan maize stem borers. *Friesen, DK and AFE. Palmer (eds.)*, p.102-105.

Mugo, S., Murenga, M.G., Karaya, H., Tende, R., Taracha, C., Gichuki, S., Ininda, J., M'bijewe, K.M. & Chavangi, A. 2011. Control of *Busseola fusca* and *Chilo partellus* stem borers by *Bacillus thuringiensis* (Bt)- δ -endotoxins from Cry1Ab gene Event MON810 in greenhouse containment trials. *African Journal of Biotechnology*, 10: 4719-4724.

Murenga, M.G., Githiri, S.M., Mugo, S.N. & Olubayo, F.M. 2011. Levels of control of *Chilo partellus* stem borer in segregation tropical Bt maize populations in Kenya. *African Journal of Biotechnology*, 10: 4725-4731.

Obonyo, D.N., Lovei, G.L., Songa, J.M., Oyieke, F.A., Mugo, S.N. & Nyamasyo, G.H.N. 2008. Developmental and mortality responses of *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and

Sesamia calamistis Hampson (Lepidoptera: Noctuidae) following partial feeding on Bt-transgenic maize. *Journal of Applied Biosciences*, 11: 554-563.

Obonyo, D.N., Songa, J.M., Oyieke, F.A., Nyamasyo, G.H.N. & Mugo, S.N. 2008. Bt-transgenic maize does not deter oviposition by two important African cereal stem borers, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Journal of Applied Biosciences*, 10: 424-433.

Prütz, G. & Dettner, K. 2004. Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions. *Entomologia Experimentalis et Applicata*, 111: 179-187.

Télez-Rodríguez, P., Raymond, B., Moran-Bertot, I, Rodríguez-Cabera, L., Wright, D.J., Borotto, C.G. & Ayra-Pardo, C. 2014. Strong oviposition preference for Bt over non-Bt maize in *Spodoptera frugiperda* and its implications for the evolution of resistance. *BMC Biology*, 12: 1-10.

Tefera, T., Mugo, S., Mwimali, M., Anani, B., Tende, R., Beyene, Y., Gichuki, S., Oikeh, S.O., Nang'ayo, F., Okeno, J., Njeu, E., Pillay, K., Meisel, B. & Prasanna, B.M. 2016. Resistance of Bt-maize (MON810) against the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) and its yield performance in Kenya. *Crop Protection*, 89: 202-208.

Tende, R.M., Nderitu, J.H., Mugo, S., Songa, J.M., Olubayo, F. & Bergvinson, D. 2005. Screening for development of resistance by the spotted stemborer *Chilo partellus* (Lepidoptera: Pyralidae) to Bt-maize delta-endotoxins. *African Crop Science Conference Proceedings*, 7: 1241-1244.

Tende, R.M., Mugo, S.N., Nderitu, J.H., Olubayo, F.M., Songa, J.M. & Bergvinson, D.J. 2010. Evaluation of *Chilo partellus* and *Busseola fusca* susceptibility to δ -endotoxins in Bt maize. *Crop Protection*, 29: 115-120.

Van Rensburg, J.B.J. 1999. Evaluation of Bt.-Transgenic maize for the resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South African Journal of Plant and Soil*, 16: 38-43.